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=> d his ful

     FILE 'HCAPLUS' ENTERED AT 10:13:02 ON 30 SEP 2004
                ACT LUC496L18/A
                ---------
L1   (          1) SEA ABB=ON  "BORDETELLA PERTUSSIS TOXIN"/CN
L2   (          1) SEA ABB=ON  "SULFATE ION"/CN
L3   (          2) SEA ABB=ON  CYSTEINE/CN
L4   (     568646) SEA ABB=ON  L1 OR ?PERTUS?(W)?TOXIN? OR PT OR ?PERTACTIN? OR
                   (B OR ?BORDELLA?)(W)(?PERTUSSIS? OR ?BRONCHISEPTICA?) OR
                   ?CLOSTRIDIUM? OR ?STAPHYLOCOCCUS? OR ?SALMONELLA? OR ?SHIGELLA?
                    OR ?VIBRIO? OR ?ESCHERICHIA?
L5   (     298936) SEA ABB=ON  L4 AND (?PRODUC? OR ?PREP? OR ?SYNTH? OR ?PURIF?
                   OR (?ENHANC? OR ?INCREAS? OR ?IMPROV? OR ?PROPAGAT? OR
                   ?MULTIPL?)(W)(?PRODUC? OR ?PREP? OR ?SYNTH? OR ?PURIF?))
L6   (       3048) SEA ABB=ON  L5 AND (L2 OR L3 OR ?SULFAT?(W)ION? OR ?CYSTEINE?)(
                   L)(?DEFIC? OR ?REDUC? OR ?LESS? OR ?MINIMIZ? OR ?INHIBIT? OR
                   ?SUPPRES? OR ?ELIM?)
L7   (        425) SEA ABB=ON  L6 AND (?METHOD? OR ?TECHNIQ? OR ?PROCED?)
L8   (        403) SEA ABB=ON  L7 AND (L3 OR ?CYSTEINE?)
L9             64 SEA ABB=ON  L8 AND (B OR ?BORDETELLA?)
                  ---------
L10            10 SEA ABB=ON  L9 AND (?MEDIUM? OR ?CULTURE?)
L11            64 SEA ABB=ON  L9 OR L10
L12           *54 SEA ABB=ON  L11 AND (PD<20000404 OR PRD<20000404)

     FILE 'REGISTRY' ENTERED AT 10:23:41 ON 30 SEP 2004
                E BORDETELLA PERTUSSIS TOXIN/CN
L13             1 SEA ABB=ON  "BORDETELLA PERTUSSIS TOXIN"/CN
                E CYSTEINE/CN
L14             2 SEA ABB=ON  CYSTEINE/CN

     FILE 'HCAPLUS' ENTERED AT 10:24:12 ON 30 SEP 2004
L15            50 SEA ABB=ON  (L13 OR ?BORDETELLA?(W)?PERTUSSIS?) AND (L14 OR
                   ?CYSTEINE?)
L16            34 SEA ABB=ON  L15 AND (?PRODUC? OR ?MANUF? OR ?PREP? OR ?SYNTH?)
L17            15 SEA ABB=ON  L16 AND (?METHOD? OR ?TECHNIQ? OR ?PROCED? OR
                   ?PROCES?)
L18            34 SEA ABB=ON  L16 OR L17 34 cits from APlus - attached
                 *SAV L12 LUC496L12/A

     FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO' ENTERED AT
     10:26:55 ON 30 SEP 2004
L19            18 SEA ABB=ON  L17
L20            14 DUP REMOV L19 (4 DUPLICATES REMOVED) 14 cits from other
                                                       databases - attached
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* These are the results from the more general search. I have saved them in case you would like to see additional citations. Just let me know!

Mary Jane

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=> d que stat l18
L13           1 SEA FILE=REGISTRY ABB=ON  "BORDETELLA PERTUSSIS TOXIN"/CN
L14           2 SEA FILE=REGISTRY ABB=ON  CYSTEINE/CN
L15          50 SEA FILE=HCAPLUS ABB=ON   (L13 OR ?BORDETELLA?(W)?PERTUSSIS?)
                AND (L14 OR ?CYSTEINE?)
L16          34 SEA FILE=HCAPLUS ABB=ON   L15 AND (?PRODUC? OR ?MANUF? OR
                ?PREP? OR ?SYNTH?)
L17          15 SEA FILE=HCAPLUS ABB=ON   L16 AND (?METHOD? OR ?TECHNIQ? OR
                ?PROCED? OR ?PROCES?)
L18          34 SEA FILE=HCAPLUS ABB=ON   L16 OR L17

=> d ibib abs l18 1-34

L18  ANSWER 1 OF 34  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:        2003:191355  HCAPLUS
DOCUMENT NUMBER:         138:381847
TITLE:                   Reduced glutathione is required for pertussis toxin
                         secretion by Bordetella pertussis
AUTHOR(S):               Stenson, Trevor H.; Patton, Angela K.; Weiss, Alison
                         A.
CORPORATE SOURCE:        Department of Molecular Genetics, Biochemistry, and
                         Microbiology, University of Cincinnati, Cincinnati,
                         OH, 45267-0524, USA
SOURCE:                  Infection and Immunity (2003), 71(3), 1316-1320
                         CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER:               American Society for Microbiology
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   The abilities of cysteine-containing compds. to support growth of
     Bordetella pertussis and influence pertussis toxin
     transcription, assembly, and secretion were examined Cysteine is
     an essential amino acid for B. pertussis and must be present for protein
     synthesis and bacterial growth. However, cysteine can
     be metabolized to sulfate, and high concns. of sulfate can selectively
     inhibit transcription of the virulence factors, including pertussis toxin,
     via the BvgAS two-component regulatory system in a process
     called modulation. In addition, pertussis toxin possesses several disulfide
     bonds, and the cysteine-containing compound glutathione can influence
     oxidation-reduction reactions and perhaps disulfide bond formation. Bacterial
     growth was not observed in the absence of a source of cysteine.
     Oxidized glutathione, as a sole source of cysteine, also did not
     support bacterial growth. Cysteine, cystine, and reduced
     glutathione did support bacterial growth, and none of these compds. caused
     modulation at the concns. tested. Similar amts. of periplasmic pertussis
     toxin were detected regardless of the source of cysteine;
     however, in the absence of reduced glutathione, pertussis toxin was not
     efficiently secreted. Addition of the reducing agent dithiothreitol was
     unable to compensate for the lack of reduced glutathione and did not
     promote secretion of pertussis toxin. These results suggest that reduced
     glutathione does not affect the accumulation of assembled active pertussis
     toxin in the periplasm but plays a role in efficient pertussis toxin
     secretion by the bacterium.
REFERENCE COUNT:         31    THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS
                               RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18  ANSWER 2 OF 34  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:        2002:753680  HCAPLUS
DOCUMENT NUMBER:         138:23481
TITLE:                   The immunomodulatory actions of prostaglandin E2 on
                         allergic airway responses in the rat
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AUTHOR(S): Martin, James G.; Suzuki, Masaru; Maghni, Karim; Pantano, Rosa; Ramos-Barbon, David; Ihaku, Daizo; Nantel, Francois; Denis, Danielle; Hamid, Qutayba; Powell, William S.
CORPORATE SOURCE: Meakins Christie Laboratories, Department of Medicine, McGill University, Montreal, QC, H2X2P2, Can.
SOURCE: Journal of Immunology (2002), 169(7), 3963-3969
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB PGE2 has been reported to inhibit allergen-induced airway responses in sensitized human subjects. The aim of this study was to investigate the mechanism of anti-inflammatory actions of PGE2 in an animal model of allergic asthma. BN rats were sensitized to OVA using **Bordetella pertussis** as an adjuvant. One week later, an aerosol of OVA was administered. After a further week, animals were anesthetized with urethane, intubated, and subjected to measurements of pulmonary resistance (RL) for a period of 8 h after OVA challenge. PGE2 (1 and 3 μg in 100 μl of saline) was administered by insufflation intratracheally 30 min before OVA challenge. The early response was inhibited by PGE2 (3 μg). The late response was inhibited by both PGE2 (1 and 3 μg). Bronchoalveolar lavage fluid from OVA-challenged rats showed eosinophilia and an increase in the number of cells expressing IL-4 and IL-5 mRNA. These responses were inhibited by PGE2. Bronchoalveolar lavage fluid levels of cysteinyl-leukotrienes were elevated after OVA challenge and were reduced after PGE2 to levels comparable with those of sham challenged animals. We conclude that PGE2 is a potent anti-inflammatory agent that may act by reducing allergen-induced Th2 cell activation and cysteinyl-leukotriene **synthesis** in the rat.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 3 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:555756 HCAPLUS
DOCUMENT NUMBER: 137:121864
TITLE: Biosensor with covalently attached membrane-spanning proteins
INVENTOR(S): Lakey, Jeremy Hugh
PATENT ASSIGNEE(S): Newcastle University Ventures Limited, UK
SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002057780	A1	20020725	WO 2002-GB222	20020118

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

EP 1352245	A1	20031015	EP 2002-732154	20020118
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2004096895	A1	20040520	US 2003-250682	20031017
PRIORITY APPLN. INFO.:			GB 2001-1279	A 20010118
			GB 2001-8947	A 20010410
			WO 2002-GB222	W 20020118

AB The invention concerns a **product** comprising: a membrane-spanning protein; a lipid membrane formed from amphiphilic mols. and membrane-spanning protein mols.; and a substrate: characterized in that the membrane protein is directly coupled to the substrate. The invention also provides a **method** for **producing** such a **product** which (i) comprises treating a substrate with a hydrophilic coating agent; (ii) providing at least one membrane-spanning protein; (iii) bringing the protein into contact with the treated substrate under conditions for the coupling of the protein directly to the treated substrate; (iv) adding amphiphilic mols. to the protein-coupled substrate to form a lipid membrane. The **product** is useful for biosensors, protein arrays and the like.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 4 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2002:123051 HCAPLUS
DOCUMENT NUMBER: 136:166156
TITLE: Purification of Hepatitis B Virus antigens for use in vaccines
INVENTOR(S): De Heyder, Koen; Schu, Peter; Serantoni, Michelle; Van Opstel, Omer
PATENT ASSIGNEE(S): Smithkline Beecham Biologicals SA, Belg.
SOURCE: PCT Int. Appl., 37 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002012287	A1	20020214	WO 2001-EP9100	20010807
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2001082073	A5	20020218	AU 2001-82073	20010807
EP 1307473	A1	20030507	EP 2001-960630	20010807
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
BR 2001013155	A	20030708	BR 2001-13155	20010807
JP 2004505992	T2	20040226	JP 2002-518258	20010807
NZ 524012	A	20040227	NZ 2001-524012	20010807
NO 2003000635	A	20030401	NO 2003-635	20030207
BG 107545	A	20040130	BG 2003-107545	20030207
US 2003235590	A1	20031225	US 2003-344211	20030718
PRIORITY APPLN. INFO.:			GB 2000-19728	A 20000810

GB 2001-1334 A 20010118
WO 2001-EP9100 W 20010807

AB The present invention relates to a **method** for the **prodn** . of a Hepatitis B antigen suitable for use in a vaccine, the **method** comprising purification of the antigen in the presence of **cysteine**, to vaccines comprising such antigens. Preferably, the invention provides a **method** for the purification of recombinant Hepatitis B antigen that **produces** a stable antigen with out a trace of thiomersal or mercury compds. which result from thiomersal decomposition Thus, thiomersal, which is included as a preservative, is excluded from Sepharose 4B gel permeation chromatog. of partially purified Hepatitis B antigen. The eluate from gel permeation chromatog. is then further purified by anion exchange chromatog. followed by d. gradient ultracentrifugation in CsCl. It was found omitting thiomersal from the purification scheme may result in the aggregation of the antigen during the d. gradient ultracentrifugation. This aggregation is then prevented by the inclusion of a suitable reducing agent to the eluate from anion exchange chromatog. prior to the d. gradient ultracentrifugation step.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 5 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:827946 HCAPLUS
DOCUMENT NUMBER: 136:323756
TITLE: Fasciola hepatica cathepsin L **cysteine** proteinase suppresses **Bordetella pertussis**-specific interferon-γ **production** in vivo
AUTHOR(S): O'Neill, Sandra M.; Mills, Kingston H. G.; Dalton, John P.
CORPORATE SOURCE: Molecular Parasitology, School of Biotechnology, Dublin City University, Dublin, Ire.
SOURCE: Parasite Immunology (2001), 23(10), 541-547
CODEN: PAIMD8; ISSN: 0141-9838
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have previously demonstrated that Fasciola hepatica infection significantly reduced **Bordetella pertussis**-specific interferon (IFN)-γ **production** in mice coinfected with B. pertussis or immunized with a pertussis whole cell vaccine (Pw). In the present study, we have identified parasite mols. capable of mimicking this suppressive effect of F. hepatica. Parenteral injection of mice with culture medium in which adult F. hepatica were maintained (excretory/secretory, ES, **products**) suppressed B. pertussis-specific IFN-γ **production** in mice immunized with Pw. The suppressive effect of ES was abrogated by coinjecting ES with the **cysteine** proteinase inhibitor, Z-Phe-Ala-diazo-methylketone. Furthermore, purified cathepsin L proteinase (FheCL), a major component of ES **products**, was capable of suppressing IFN-γ **production** The suppressive effect of FheCL was attenuated in interleukin (IL)-4 defective (IL-4-1-) mice. Therefore, FheCL released by F. hepatica is involved in the suppression of Th1 immune responses and this suppression may be dependent upon IL-4.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 6 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:798235 HCAPLUS
DOCUMENT NUMBER: 135:339212

TITLE: The use of azalide antibiotic compositions for treating or preventing a bacterial or protozoal infection in mammals
INVENTOR(S): Boettner, Wayne Alan; Canning, Peter Connor
PATENT ASSIGNEE(S): Pfizer Products Inc., USA
SOURCE: PCT Int. Appl., 74 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001081358	A1	20011101	WO 2001-IB519	20010326
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1276747	A1	20030122	EP 2001-915612	20010326
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
BR 2001010382	A	20030624	BR 2001-10382	20010326
JP 2004516233	T2	20040603	JP 2001-578446	20010326
US 2002019353	A1	20020214	US 2001-829672	20010410
BG 107168	A	20030731	BG 2002-107168	20021003
ZA 2002008603	A	20031024	ZA 2002-8603	20021024
NO 2002005134	A	20021219	NO 2002-5134	20021025
PRIORITY APPLN. INFO.:			US 2000-199961P P	20000427
			WO 2001-IB519 W	20010326

OTHER SOURCE(S): MARPAT 135:339212

AB **Methods** for treating or preventing bacterial or protozoal infections in mammals by administering a single dose of an antibiotic composition comprising a mixture of azalide isomers and a pharmaceutically acceptable vehicle are disclosed. **Methods** for increasing acute or chronic injection-site toleration in mammals by administering a single dose of antibiotic compns. comprising a mixture of azalide isomers and a pharmaceutically acceptable vehicle are also disclosed. A combination comprising an antibiotic composition comprising a mixture of azalide isomers, a pharmaceutically acceptable carrier, and instructions for use in a single-dose administration is also disclosed.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 7 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:790967 HCAPLUS
DOCUMENT NUMBER: 136:66733
TITLE: **Bordetella pertussis** autoregulates pertussis toxin **production** through the metabolism of **cysteine**
AUTHOR(S): Bogdan, John A.; Nazario-Larrieu, Javier; Sarwar, Jawad; Alexander, Peter; Blake, M. S.
CORPORATE SOURCE: Baxter Healthcare Corporation, Columbia, MD, 21046-2358, USA
SOURCE: Infection and Immunity (2001), 69(11), 6823-6830

CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Pertussis toxin (Ptx) expression and secretion in B. pertussis are regulated by a 2-component signal transduction system encoded by the bvg regulatory locus. However, it is not known whether the metabolic pathways and growth state of the bacterium influence **synthesis** and secretion of Ptx and other virulence factors. We have observed a reduction in the concentration of Ptx per optical d. unit midway in fermentation Studies were conducted to identify possible factors causing this reduction and to develop culture conditions that optimize Ptx expression. Medium reconstitution expts. demonstrated that spent medium and a fraction of this medium containing components with a mol. weight of <3,000 inhibited the **production** of Ptx. A complete flux anal. of the intermediate metabolism of B. pertussis revealed that the S-containing amino acids methionine and **cysteine** and the organic acid pyruvate accumulated in the media. In fermentation, a large amount of internal SO42- was observed in early stage growth, followed by a rapid decrease as the cells entered into logarithmic growth. This loss was later followed by the accumulation of large quantities of SO42- into the media in late-stage fermentation Release of SO42- into the media by the cells signaled the decoupling of cell growth and Ptx **production** Under conditions that limited **cysteine**, a 5-fold increase in Ptx **production** was observed Addition of BaCl2 to the culture further increased Ptx yield. These results suggest that B. pertussis is capable of autoregulating the activity of the bvg regulon through its metabolism of **cysteine.** Reduction of the amount of **cysteine** in the media results in prolonged vir expression due to the absence of the neg. inhibitor SO42-. Therefore, the combined presence and metabolism of **cysteine** may be an important mechanism in the pathogenesis of B. pertussis.

REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 8 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:747833 HCAPLUS
DOCUMENT NUMBER: 135:302952
TITLE: Improved **method** for the **production** of bacterial toxins
INVENTOR(S): Blake, Milan S.; Bogdan, John A., Jr.; Nazario-Larrieu, Javier
PATENT ASSIGNEE(S): Baxter International Inc., USA; Baxter Healthcare S.A.
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001074862	A2	20011011	WO 2001-US10938	20010404
WO 2001074862	A3	20021003		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN,

YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2002061555	A1	20020523	US 2001-825770	20010404
US 6686180	B2	20040203		
US 2002165344	A1	20021107	US 2001-825769	20010404
EP 1268531	A2	20030102	EP 2001-926612	20010404
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2003531586	T2	20031028	JP 2001-572551	20010404
PRIORITY APPLN. INFO.:			US 2000-194478P P	20000404
			US 2000-194482P P	20000404
			WO 2001-US10938 W	20010404

AB **Methods** and compns. are provided for the enhanced **prodn**. of bacterial toxins in large-scale cultures. Specifically, **methods** and compns. for reducing bacterial toxin expression inhibitors are provided including, but not limited to, addition of toxin expression inhibitor binding compds., culture media having reduced concns. of toxin inhibitor metabolic precursors and genetically modified toxigenic bacteria lacking enzymes required to metabolize the toxin inhibitor metabolic precursors.

L18 ANSWER 9 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	2001:716904 HCAPLUS
DOCUMENT NUMBER:	137:105879
TITLE:	A quantitative analysis for the ADP-Ribosylation activity of pertussis toxin: An enzymatic-HPLC coupled assay applicable to formulated whole cell and acellular pertussis vaccine **products**
AUTHOR(S):	Cyr, Terry; Menzies, Allan J.; Calver, Jerry; Whitehouse, Larry W.
CORPORATE SOURCE:	Research Services Division, Health Products and Food Branch, Health Canada, Ottawa, K1A 0L2, Can.
SOURCE:	Biologicals (2001), 29(2), 81-95 CODEN: BILSEC; ISSN: 1045-1056
PUBLISHER:	Academic Press
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB The majority of the biol. effects of pertussis toxin (PT) are the result of a toxin-catalyzed transfer of an ADP-ribose (ADP-ribose) moiety from NAD+to the α-subunits of a subset of signal-transducing guanine-nucleotide-binding proteins (G-proteins). This generally leads to an uncoupling of the modified G-protein from the corresponding receptor and the loss of effector regulation. This assay is based on the PT S1 subunit enzymic transfer of ADP-ribose from NAD to the **cysteine** moiety of a fluorescent tagged **synthetic** peptide homologous to the 20 amino acid residue carboxyl-terminal sequence of the α-subunit of the Gi3protein. The tagged peptide and the ADP-ribosylated **product** were characterized by HPLC/MS and MS/MS for structure confirmation. Quantitation of this characterized ADP-ribosylated fluorescently tagged peptide was by HPLC fluorescence using Standard Addition **methodol.** The assay was linear over a five hr incubation period at 20° at PT concns. between 0·0625 and 4·0 μg/mL and the sensitivity of the assay could be increased several fold by increasing the incubation time to 24 h. Purified S1 subunit of PT exhibited 68·1±10·1% of the activity of the intact toxin on a molar basis, whereas the pertussis toxin B oligomer, the genetically engineered toxoid, (PT-9K/129G), and several of the other components of the **Bordetella pertussis** organism

possessed little (<0·6%) or no detectable ribosylation activity. Commonly used pertussis vaccine reference materials, US PV Lot 11, BRP PV 66/303, and BRP PV 88/522, were assayed by this **method** against **Bordetella pertussis** Toxin Standard 90/518 and demonstrated to contain, resp., 0·323±0·007, 0·682.+-.0·045, and 0·757±0·006 μg PT/mL (Mean±SEM) or in terms of μg/vial: 3·63, 4·09 and 4·54, resp. A survey of several multivalent pertussis vaccine **products** formulated with both whole cell as well as acellular components indicated that **products** possessed a wide range of ribosylation activities. The pertussis toxin S1 subunit catalyzed ADP- ribosylation of the FAC-Gαi3C20 peptide substrate and its subsequent quantitation by HPLC was demonstrated to be a sensitive and quant. **method** for measuring intrinsic pertussis toxin activity. This **methodol.** not only has the potential to be an alternative physicochem. **method** to replace existing bioassay **methodol.**, but has the added advantage of being a universal **method** applicable to the assay of pertussis toxin in both whole cell and acellular vaccines as well as bulk and final formulated vaccine **products.** Acceptance of this **method** by regulatory agencies and industry as a credible alternative to existing **methods** would, however, require validation in an international collaborative study against the widely accepted bioassay **methods.** (c) 2001 The International Association of Biological Standardization.

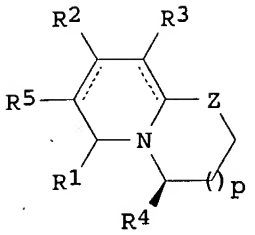
REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 10 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:380591 HCAPLUS
DOCUMENT NUMBER: 135:5629
TITLE: **Synthesis** and use of substituted pyridinones to treat and prevent bacterial infections
INVENTOR(S): Almqvist, Fredrik; Emtenas, Hans; Hultgren, Scott J.; Pinkner, Jerome S.
PATENT ASSIGNEE(S): Washington University, USA
SOURCE: PCT Int. Appl., 134 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

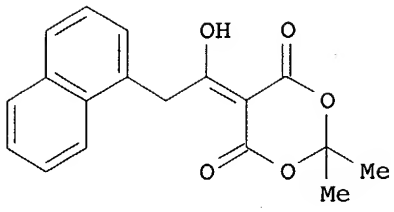
PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001036426	A1	20010525	WO 2000-US31879	20001120
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1233967	A1	20020828	EP 2000-982170	20001120
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: US 1999-166621P P 19991119
WO 2000-US31879 W 20001120
OTHER SOURCE(S): CASREACT 135:5629; MARPAT 135:5629

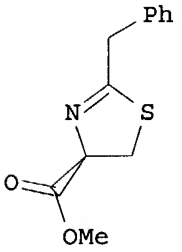
GI



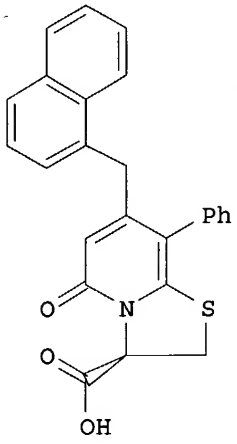
I



III



IV



V

AB Pyridinones I (unsatd.; p = 1 or 0) and I (saturated; p = 1; II), a **process** for their **preparation** and use as antibacterials are claimed; [wherein; Z = S(O)0-2, O, P(O)0-2, CH2 or CR2; R1 = oxo; R2 = (CH2)0-5A where A is H, (substituted)alk(en/yn)yl, or (substituted)(hetero)aryl; R3 = (CH2)0-5D where D = A; R4 = CO2Y, B(OY)2, CHO, CH2OY, CH(CO2Y)2, PO(OY)2 where Y = A; R5 = H, halo, CN, CO2H, CH2NH2, cyclic CHN4, NO2, (TMS)acetylene, alkenyl, etc.]. Several **synthetic** examples are provided. An example of the **process** claimed is represented by the regioselective reaction of III with (**cysteine** derived) thiazoline IV (or the corresponding 6-membered ring imine) in a solvent at 5°-15°C in the presence of a Lewis/hydrochloric acid followed by heating to 50°-70°C which **produces** the Me ester derivative of acid V. An analogous **process** utilizing a polymer-bound analog of IV is also claimed. Liberation of the ester/polymer-bound adduct by saponification **produces** V. Elaboration of pyridinones I (R5 = H) is accomplished by electrophilic substitution (to R5 = halo, NO2, etc.) and the resulting halo derivs. subjected to Pd-catalyzed coupling (to R5 = CN, vinyl, (TMS)acetylenyl, etc.). Pyridinone I (R5 = CN; p = 1) may be further transformed into I (R5 = -CH2NH2, tetrazole, COOH, etc.). Derivs. II are **prepared** by Pt or Pd-mediated reduction of compds. I. Compds. of the invention are antibacterial agents. Selected compds. were evaluated for binding to chaperone proteins PapD and FimC. Inhibition of colonization and prevention/treatment of infections of Gram-neg. organisms are claimed uses of pyridinones I.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 11 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:283986 HCAPLUS
DOCUMENT NUMBER: 134:309693
TITLE: AB5 toxin B subunit mutants with altered chemical conjugation characteristics
INVENTOR(S): Handley, Harold H.; Haaparanta, Tapio; Ewalt, Karla L.
PATENT ASSIGNEE(S): Active Biotech AB, Swed.
SOURCE: PCT Int. Appl., 77 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001027144	A2	20010419	WO 2000-US27607	20001005
WO 2001027144	A3	20020117		
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1222202	A2	20020717	EP 2000-968795	20001005
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
JP 2003511061	T2	20030325	JP 2001-530362	20001005
NZ 518342	A	20040430	NZ 2000-518342	20001005
PRIORITY APPLN. INFO.:			US 1999-158561P	P 19991008
			WO 2000-US27607	W 20001005

AB A recombinant AB5 B subunit protein including at least one mutation, wherein the mutation alters the number of amino acid residues available for chemical modification as compared to a wild type AB5 B subunit protein, and wherein said recombinant protein retains an effective target ligand bind affinity. For example, specifically designed mutations are **produced** in the cholera Toxin B subunit (CTB) such that it can still bind with high affinity to its receptor, Gm-1, but can be specifically covalently linked at lysines or **cysteines** to an immunogen or vaccine. The vaccine **produced** from this coupling is a mucosal vaccine which has high immunogenicity due to the interaction with the CTB. The vaccine can be **produced** inexpensively and easily. Alternatively, a **technique** is disclosed for treating CTB such that non-covalent coupling to a vaccine or immunogen can occur. The disclosed CTB can not only be used as vaccine but also as bioactive mol. delivery agent.

L18 ANSWER 12 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:114938 HCAPLUS
DOCUMENT NUMBER: 134:173013
TITLE: Anti-bacterial compounds directed against pilus biogenesis, adhesion and activity; co-crystals of pilus subunits and **methods** of use thereof
INVENTOR(S): Hultgren, Scott J.; Sauer, Frederic G.; Waksman,

Gabriel; Fuetterer, Klaus
PATENT ASSIGNEE(S): Washington University, USA
SOURCE: PCT Int. Appl., 144 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001010386	A2	20010215	WO 2000-US22087	20000811
WO 2001010386	A3	20010802		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000074703	A5	20010305	AU 2000-74703	20000811
PRIORITY APPLN. INFO.:			US 1999-148280P P	19990811
			WO 2000-US22087 W	20000811

OTHER SOURCE(S): MARPAT 134:173013

AB Many Gram-neg. pathogens assemble adhesive structures on their surfaces that allow them to colonize host tissues and cause disease. Novel compns. for the prevention or inhibition of pilus assembly in Gram-neg. pathogens are disclosed. Interacting with the binding site of pili subunits will neg. affect the chaperone/usher pathway which is one mol. mechanism by which Gram-neg. bacteria assemble adhesive pili structures and thus prevent or inhibit pilus assembly. Addnl., novel compds. and compns. for interfering or preventing adhesion of pileated bacteria to host tissues are provided. Such compds. and compns. prevent or inhibit pili adhesion to host tissues by interacting with the mannose-binding domains on pilus adhesin subunits. Also provided are **methods** for the treatment or prevention of diseases caused by tissue-adhering pilus-forming bacteria by interaction with the binding between pilus subunits; the binding between pilus subunits and periplasmic chaperones; and the binding of a pilus adhesin to the host epithelial tissue. Also provided are pharmaceutical **prepns.** capable of interacting with the binding between pilus subunits, between pilus subunits and periplasmic chaperones and between the pilus adhesin. The present invention further relates to co-crystals of pilus chaperone-subunit co-complexes, detailed three dimensional structural information illustrating the interaction between pilus subunits and/or between a pilus subunit and a chaperone for a pilus chaperone-subunit co-complex and **methods** of utilizing the X-ray crystallog. data from such co-crystals to design, identify and screen for compds. that exhibit antibacterial activity. The present invention also relates to machine readable media embedded with the three-dimensional atomic structure coordinates of pilus chaperone-subunit co-complex and subsets thereof.

L18 ANSWER 13 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:889102 HCAPLUS
DOCUMENT NUMBER: 135:72024
TITLE: Four genes are required for the system II cytochrome c biogenesis pathway in **Bordetella pertussis**, a unique bacterial model

AUTHOR(S): Beckett, Caroline S.; Loughman, Jennifer A.; Karberg, Katherine A.; Donato, Gina M.; Goldman, William E.; Kranz, Robert G.
CORPORATE SOURCE: Department of Biology, Washington University, St Louis, MO, 63130, USA
SOURCE: Molecular Microbiology (2000), 38(3), 465-481
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Unlike other cytochromes, c-type cytochromes have two covalent bonds formed between the two vinyl groups of haem and two **cysteines** of the protein. This haem ligation requires specific assembly proteins in prokaryotes or eukaryotic mitochondria and chloroplasts. Here, it is shown that **Bordetella pertussis** is an excellent bacterial model for the widespread system II cytochrome c **synthesis** pathway. Mutations in four different genes (ccsA, ccsB, ccsX and dipZ) result in B. pertussis strains unable to **synthesize** any of at least seven c-type cytochromes. Using a cytochrome c4:alkaline phosphatase fusion protein as a bifunctional reporter, it was demonstrated that the B. pertussis wild-type and mutant strains secrete an active alkaline phosphatase fusion protein. However, unlike the wild type, all four mutants are unable to attach haem covalently, resulting in a degraded N-terminal apocytochrome c4 component. Thus, apocytochrome c secretion is normal in each of the four mutants, but all are defective in a periplasmic assembly step (or export of haem). CcsX is related to thioredoxins, which possess a conserved CysXxxXxxCys motif. Using phoA gene fusions as reporters, CcsX was proven to be a periplasmic thioredoxin-like protein. Both the B. pertussis dipZ(i.e. dsbD) and ccsX mutants are corrected for their assembly defects by the thiol-reducing compds., dithiothreitol and 2-mercaptoethanesulfonic acid. These results indicate that DipZ and CcsX are required for the periplasmic reduction of the **cysteines** of apocytochromes c before ligation. In contrast, the ccsA and ccsB mutants are not corrected by exogenous reducing agents, suggesting that CcsA and CcsB are required for the haem ligation step itself in the periplasm (or export of haem to the periplasm). Related to this suggestion, the topol. of CcsB was determined exptl., demonstrating that CcsB has four transmembrane domains and a large 435-amino-acid periplasmic region.

REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 14 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:294247 HCAPLUS
DOCUMENT NUMBER: 132:307364
TITLE: Culture medium for Bordetella and cultivation of the bacteria to **produce** pertussis toxins, etc.
INVENTOR(S): Takisawa, Kazuyuki; Kurosawa, Daisuke; Maruyama, Hiroichi; Sakai, Nobuo; Ikushima, Koichiro; Sato, Masaya
PATENT ASSIGNEE(S): Denka Seiken K. K., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2000125852	A2	20000509	JP 1998-298674	19981020

PRIORITY APPLN. INFO.: JP 1998-298674 19981020

AB The medium contains polyethylene glycol (I) or its lower alkyl ethers, preferably with average mol. weight 2000-400,000 and optionally D-glucose polymer ethers and cyclodextrin. Bordetella is cultured in the medium to **produce** pertussis toxin and filamentous hemagglutinin useful for vaccine **production** at higher yields. Addition of I to a medium containing casamino acid, yeast extract, L-**cysteine** hydrochloride, niacin, and salts in cultivation of B. pertussis Tohama I increased yield of the toxin and the hemagglutinin from 280 and 40 EU/mL, resp., to 480 and 150 EU/mL, resp. **Production** of the hemagglutinin was further increased to 1250 EU/mL when Me cellulose was used in addition to I.

L18 ANSWER 15 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:265485 HCAPLUS

DOCUMENT NUMBER: 131:40796

TITLE: The conserved lysine 860 in the additional fatty-acylation site of **Bordetella pertussis** adenylate cyclase is crucial for toxin function independently of its acylation status

AUTHOR(S): Basar, Tumay; Havlicek, Vladimir; Bezouskova, Silvia; Halada, Petr; Hackett, Murray; Sebo, Peter

CORPORATE SOURCE: Institute of Microbiology of the Academy of Sciences of the Czech Republic, Prague, CZ-142 20/4, Czech Rep.

SOURCE: Journal of Biological Chemistry (1999), 274(16), 10777-10783
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **Bordetella pertussis** RTX (repeat in toxin family protein) adenylate cyclase toxin-hemolysin (ACT) acquires biol. activity upon a single amide-linked palmitoylation of the ε-amino group of lysine 983 (Lys983) by the accessory fatty-acyltransferase CyaC. However, an addnl. conserved RTX acylation site can be identified in ACT at lysine 860 (Lys860), and this residue becomes palmitoylated when recombinant ACT (r-Ec-ACT) is **produced** together with CyaC in Escherichia coli K12. We have eliminated this addnl. acylation site by replacing Lys860 of ACT with arginine, leucine, and **cysteine** residues. Two-dimensional gel electrophoresis and microcapillary high performance liquid chromatog./tandem mass spectrometric analyses of mutant proteins confirmed that the two sites are acylated independently in vivo and that mutations of Lys860 did not affect the quant. acylation of Lys983 by palmitoyl (C16:0) and palmitoleil (cis Δ9 C16:1) fatty-acyl groups. Nevertheless, even the most conservative substitution of lysine 860 by an arginine residue caused a 10-fold decrease of toxin activity. This resulted from a 5-fold reduction of cell association capacity and a further 2-fold reduction in cell penetration efficiency of the membrane-bound K860R toxin. These results suggest that lysine 860 plays by itself a crucial structural role in membrane insertion and translocation of the toxin, independently of its acylation status.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 16 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1998:621228 HCAPLUS

DOCUMENT NUMBER: 129:240866

TITLE: Positive-selection cloning vectors using a resistance

marker containing an intein sequence to identify open reading frames
INVENTOR(S): Jacobs, William R.; Daugelat, Sabine
PATENT ASSIGNEE(S): Albert Einstein College of Medicine of Yeshiva University, USA
SOURCE: PCT Int. Appl., 83 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9840394	A1	19980917	WO 1998-US4805	19980310
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5981182	A	19991109	US 1997-816721	19970313
AU 9869389	A1	19980929	AU 1998-69389	19980310
PRIORITY APPLN. INFO.:			US 1997-816721	19970313
			WO 1998-US4805	19980310

AB Cloning vectors that make use of the self-excising properties of inteins to identify open reading frames are described. An intein is excised from a larger protein providing certain minimal sequence requirements around the excision sites are met. The remainder of the intein may include a foreign protein. If the intein is introduced into a resistance marker, then successful self-excision will lead to the development of resistance. If a sequence that is not an open reading frame is cloned into the intein sequence, then the resistance marker **product** will not be formed and the organism carrying the sequence will be sensitive to the selective agent. The vectors include a cloning site in the intein coding region, and appropriate promoters and replication origins. The vector constructs of the present invention may contain DNA of interest cloned into a unique restriction site of the intein, and may be used as a vaccine alone or transformed into a vaccine vector. In particular, these vectors are intended for use in the cloning of sequences encoding protective antigens. The use of the intein of the Mycobacterium tuberculosis recA gene in the aph (kanamycin resistance gene) is demonstrated using Escherichia coli and Mycobacterium smegmatis as hosts. When the intein can be correctly spliced, a very large fraction (>75%) of cfu's are kanamycin resistant. In constructs designed to prevent excision of the intein, the frequency of kanamycin resistant cfu's fell to as low as 1 in 4+106 in E. coli and 1 in 3+108 in M. smegmatis. Further anal. showed that splicing efficiency was very dependent upon the site used for integration of the foreign sequence. Use of the **method** to clone open reading frames from well characterized genomes (mycobacteriophage L5, Haemophilus influenzae) is demonstrated.
REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 17 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:554736 HCAPLUS
DOCUMENT NUMBER: 129:242354
TITLE: Evidence that a globular conformation is not

compatible with FhaC-mediated secretion of the **Bordetella pertussis** filamentous hemagglutinin

AUTHOR(S): Guedin, Sandrine; Willery, Eve; Locht, Camille; Jacob-Dubuisson, Francoise

CORPORATE SOURCE: INSERM U447, IBL, Institut Pasteur de Lille, Lille, 59019, Fr.

SOURCE: Molecular Microbiology (1998), 29(3), 763-774
CODEN: MOMIEE; ISSN: 0950-382X

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The 220-kDa B. pertussis filamentous hemagglutinin (FHA) is the major extracellular protein of this organism. It is exported using a signal peptide-dependent pathway, and its secretion depends on 1 specific outer membrane accessory protein, FhaC. In this work, the influence of conformation on the FhaC-mediated secretion of FHA was investigated using an 80 kDa N-terminal FHA derivative, Fha44. In contrast to many signal peptide-dependent secretory proteins, no soluble periplasmic intermediate of Fha44 could be isolated. In addition, cell-associated Fha44 **synthesized** in the absence of FhaC did not remain competent for extracellular secretion upon delayed expression of FhaC, indicating that the translocation steps across the cytoplasmic and the outer membrane might be coupled. A chimeric protein, in which the globular B subunit of the cholera toxin, CtxB, was fused at the C-terminus of Fha44, was not secreted in B. pertussis or in Escherichia coli expressing FhaC. The hybrid protein was secreted only when both disulfide bond-forming **cysteines** of CtxB were replaced by serines or when it was **produced** in DsbA E. coli. The Fha44 portion of the secretion-incompetent hybrid protein was partly exposed on the cell surface. These results argue that the Fha44-CtxB hybrid protein transited through the periplasmic space, where disulfide bond formation is specifically catalyzed, and that secretion across the outer membrane was initiated. The folded CtxB portion prevented extracellular release of the hybrid, in contrast to the more flexible CtxB domain devoid of **cysteines.** A secretion model is proposed whereby Fha44 transits through the periplasmic space on its way to the cell surface and initiates its translocation through the outer membrane before being released from the cytoplasmic membrane. Coupling of Fha44 translocation across both membranes would delay the acquisition of its folded structure until the protein emerges from the outer membrane. Such a model would be consistent with the extensive intracellular proteolysis of FHA derivs. in B. pertussis.

REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 18 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1998:439192 HCAPLUS

DOCUMENT NUMBER: 129:172840

TITLE: N-terminal characterization of the **Bordetella pertussis** filamentous hemagglutinin

AUTHOR(S): Lambert-Buisine, Corinne; Willery, Eve; Locht, Camille; Jacob-Dubuisson, Frangoise

CORPORATE SOURCE: INSERM, Institut Pasteur de Lille, Lille, 59019, Fr.

SOURCE: Molecular Microbiology (1998), 28(6), 1283-1293
CODEN: MOMIEE; ISSN: 0950-382X

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The major adhesin of **Bordetella pertussis**, filamentous

hemagglutinin (FHA), is **produced** and secreted at high levels by the bacterium. Mature FHA derives from a large precursor, FhaB, that undergoes several post-translational maturations. It was demonstrated by site-directed mutagenesis that the N-terminal signal peptide of FHA is composed of 71 amino acids, including a 22-residue-long N-terminal extension sequence. This sequence, although highly conserved in various other secretory proteins, does not appear to play an essential part in FHA secretion, as shown by deletion mutagenesis. The entire N-terminal signal region of FhaB is removed in the course of secretion by proteolytic cleavage at a site that corresponds to a Lep signal peptidase recognition sequence. After this maturation, the N-terminal glutamine residue is modified to a pyroglutamate residue. This modification is not crucial for heparin binding, hemagglutination or secretion. Interestingly, however, the modification is absent from Escherichia coli secreted FHA derivs. In addition, it is dependent in B. pertussis on the presence of all three **cysteines** contained in the signal peptide of FhaB. These observations suggest that it does not occur spontaneously but perhaps requires a specific enzymic machinery.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 19 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:648937 HCAPLUS
DOCUMENT NUMBER: 125:298006
TITLE: ADP-ribosylation of Gαi and Gαo in pituitary cells enhances their recognition by antibodies directed against their carboxyl termini
AUTHOR(S): Cussac, Didier; Kordon, Claude; Enjalbert, Alain; Saltarelli, Daniele
CORPORATE SOURCE: Faculte Medecine Secteur Nord, ICNE UMR 9941 CNRS, Marseille, F-13916, Fr.
SOURCE: Journal of Receptor and Signal Transduction Research (1996), 16(3 & 4), 169-190
CODEN: JRETET; ISSN: 1079-9893
PUBLISHER: Dekker
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Using antibodies raised against **synthetic** peptides of heterotrimeric GTP binding proteins, the authors demonstrate the presence of Gαs, Gαi1, Gαi2, Gαi3, Gαo2, and Gβ subunits in pituitary cells. Pretreatment of pituitary cells with cholera toxin diminished the immunoreactivity of Gαs and this decrease was kinetically coupled to the rate of Gαs ADP-ribosylation. ADP-ribosylation by islet activating protein (IAP or **Bordetella pertussis** toxin) of Gαi and Gαo enhanced their immunoreactivities to antibodies raised against **synthetic** decapeptides that correspond to the Gα C-termini. Such enhancement was not observed when antibodies directed against the N-termini were used. These findings are consistent with the fact that ADP-ribosylation by IAP occurs on the **cysteine** located in the C-terminal part of Gαi and Gαo. These observations mean that the kinetics and extent of Gi and Go ADP-ribosylation by IAP in whole pituitary cells and membrane **prepns.** can be followed. It could be that ADP-ribosylation causes conformational changes in Gαi and Gαo. Indeed, the authors observed that ADP-ribosylated Gαi was more sensitive to trypsin proteolysis and that the ADP-ribosylation rates of Gαi and Gαo in whole cells were comparable to the rate of loss of coupling between inhibitory neurohormone receptors and adenylyl cyclase.

L18 ANSWER 20 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1990:609990 HCAPLUS
DOCUMENT NUMBER: 113:209990
TITLE: **Biosynthesis** of lymphocytosis-promoting factor (LPF) and filamentous hemagglutinin (FHA) components of **Bordetella pertussis** in different liquid media
AUTHOR(S): Ozcengiz, Erkan; Gunalp, Ayfer
CORPORATE SOURCE: Bogmaca Asisi Uretim Arastirma Lab., Refik Saydaam Hifzissihha Merkezi Baskanligi, Ankara, Turk.
SOURCE: Doga: Turk Saglik Bilimleri Dergisi (1990), 14(2), 307-14
CODEN: DTJSEX
DOCUMENT TYPE: Journal
LANGUAGE: Turkish
AB B. pertussis was grown in Stainer-Scholte Morse-Bray and modified Morse-Bray liquid media, and growth and toxin **production** were determined Toxin **biosynthesis** started earlier and developed to a greater extent in Morse-Bray liquid medium modified by increasing the concentration of GSH and L-**cysteine** and by adding L-proline. GSH, being an organic S source, is the crucial effector of toxin **production**

L18 ANSWER 21 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:453252 HCAPLUS
DOCUMENT NUMBER: 109:53252
TITLE: **Process** for the **preparation** of extracellular antigen fractions of **Bordetella pertussis**, and vaccine against whooping-cough containing such fractions.
INVENTOR(S): Bellalou, Jacques
PATENT ASSIGNEE(S): Institut Pasteur, Fr.
SOURCE: Eur. Pat. Appl., 19 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 252838	A1	19880113	EP 1987-401586	19870707
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
FR 2601250	A1	19880115	FR 1986-9900	19860708
FR 2601250	B1	19891201		
WO 8800058	A1	19880114	WO 1987-FR264	19870707
W: DK, JP, US				
PRIORITY APPLN. INFO.:			FR 1986-9900	19860708

AB A **process** for **preparation** of extracellular antigenic fractions of Bordetalla pertussis involves (a) culture of B. pertussis in a medium which promotes secretion of the desired antigenic fractions, with injection of O near the bottom of the vessel; (b) treatment of the supernatants; and (c) extraction and purification The desired fractions are pertussis toxin (PT) and filamentous hemagglutinin (FHA); the fractions are used for pertussis vaccine. B. pertussis Was cultured in a medium containing salts, sodium L-glutamate, Casamino acids, L-proline, L-**cysteine**, glutathion, ascorbic and nicotinic acids, and Tris, for 44-48 h; O was supplied to the medium by injection near the bottom, to supply about 12 mmol O/L/h, so that O supply was non-limiting.

L18 ANSWER 22 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1985:182250 HCAPLUS
DOCUMENT NUMBER: 102:182250
TITLE: Expression of virulence determinants in **Bordetella pertussis** and Neisseria gonorrhoeae
AUTHOR(S): Robinson, A.; Gorringe, A. R.; Keevil, C. W.
CORPORATE SOURCE: Cent. Appl. Microbiol. Res., PHLS, Salisbury/Wiltshire, SP4 0JG, UK
SOURCE: Contin. Cult. 8 [Eight]: Biotechnol., Med. Environ. (1984), 22-37. Editor(s): Dean, Alastair Campbell Ross; Ellwood, D. C.; Evans, C. G. T. Horwood: Chichester, UK.
CODEN: 53MWA8
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Virulent strains of N. gonorrhoeae were grown in the chemostat for extended periods under glucose- and O-limited conditions. Virulence was, however, lost under cystine limitation. The colonial morphol. of organisms recovered from the chemostat was strain-dependent and differed from that of organisms recovered from guinea pig chambers. Fully virulent, antigenically stable B. pertussis were also grown in the chemostat. Conditions for transition from X-mode (virulent) to C-mode (avirulent) were determined The chemostat provides a means of cultural manipulation for vaccine **production**

L18 ANSWER 23 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1985:4360 HCAPLUS
DOCUMENT NUMBER: 102:4360
TITLE: **Production** of HA fraction containing protective antigens of **Bordetella pertussis** and pertussis vaccine
INVENTOR(S): Ginnaga, Akihiro; Koba, Hiroshi; Sakuma, Shin; Kitagawa, Hisashi; Yamada, Akira; Suzuki, Yoji
PATENT ASSIGNEE(S): Chemo-Sero-Therapeutic Research Institute, Japan; Teijin Ltd.
SOURCE: Eur. Pat. Appl., 35 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 121249	A2	19841010	EP 1984-103504	19840329
EP 121249	A3	19861230		
EP 121249	B1	19910710		
R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
JP 59181222	A2	19841015	JP 1983-54680	19830330
JP 01000930	B4	19890110		
JP 59184132	A2	19841019	JP 1983-58548	19830402
JP 01000931	B4	19890110		
CA 1213234	A1	19861028	CA 1984-450495	19840326
AU 8426230	A1	19841004	AU 1984-26230	19840329
AU 564634	B2	19870820		
ES 531112	A1	19850616	ES 1984-531112	19840329
SU 1447266	A3	19881223	SU 1984-3728854	19840329
AT 65028	E	19910715	AT 1984-103504	19840329
US 4687738	A	19870818	US 1986-874670	19860616

PRIORITY APPLN. INFO.:

JP 1983-54680	19830330
JP 1983-58548	19830402
US 1984-591169	19840319
EP 1984-103504	19840329

AB A **method** was developed for the **production** of antigens (hemagglutinins, HAs) of B. pertussis. B. pertussis Is inoculated into a liquid culture medium that contains cyclodextrin or its derivs. glutathione, ascorbic acid, and Casamino acids. The B. pertussia is cultured by spinner culture at 20-37° and O concentration of 0.7-6.0 ppm under defoaming conditions. The HA is harvested from the culture broth at the stage of logarithmic growth. The isolated HA is formalinized in the presence of amino acids. This vaccine can be mixed with diphtheria toxoid and tetanus toxoid to **produce** a combined vaccine.

L18 ANSWER 24 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1984:33148 HCAPLUS
DOCUMENT NUMBER: 100:33148
TITLE: Ornithine-containing lipid of **Bordetella pertussis**, a new type of hemagglutinin
AUTHOR(S): Kawai, Yohko; Yano, Ikuya
CORPORATE SOURCE: Dep. Bacteriol., Natl. Inst. Health, Tokyo, 141, Japan
SOURCE: European Journal of Biochemistry (1983), 136(3), 531-8
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The ornithine-containing lipids of 6 strains (phases I-IV) of B. pertussis were **prepared** from the total extractable cellular lipids by thin-layer chromatog. and treatment with phospholipase A. They were compared with those **prepared** from 2 strains each of B. parapertussis and B. bronchiseptica. The structures of the ornithine-containing lipid of B. pertussis and the other 2 species were resolved by acid and alkaline hydrolysis, gas-liquid chromatog., IR absorption spectroscopy, amino acid anal., and combined gas-liquid chromatog./mass spectrometry. The main structure of the aminolipid of 3 species of Bordetella was 3-hydroxyhexadecanoic acid, amide-linked to ornithine and esterified to a 2nd hexadecanoic acid. The aminolipid of B. pertussis Sakurayashiki (phase III) exhibited high hemagglutinating activity for human and rabbit erythrocytes, having a min. hemagglutinating concentration of 1 μg/mL against 8-16 μg/mL for the other strains of Bordetella. All of these aminolipids showeds some degree of microheterogeneity. Because the 3-hydroxyhexadecanoic acid content was especially high in strain Sakurayashiki, it was presumed that the intensity of hemagglutinating activity of the aminolipid was affected by the chain length of the central 3-hydroxy fatty acid, that is the aminolipid containing 3-hydroxyhexadecanoic acid had high hemagglutinating activity. The hemagglutination was inhibited by phosphatidylcholine at concns. of more than 20 μg/mL. Other inhibitory substances were **cysteine**, sphingomyelin, acidic amino acids, histidine, unsatd. fatty acid, and basic amino acids. Furthermore, the divalent cations Ca2+ and Mg2+ inhibited this hemagglutination at a concentration of 1 mM. The O-deacylated ornithine-containing lipid that had lost hexadecanoic acid did not have any hemagglutinating activity but did have hemolytic activity. Observation by electron microscopy indicated that erythrocytes were combined by the liposomes of the ornithine-containing lipids. On the basis of these results, the proposed mechanism of hemagglutination by the aminolipids is that the liposomes of the aminolipids combine erythrocytes by hydrophobic interaction between the fatty acid moieties of the aminolipid and the lipids of the surface of erythrocytes, and by ionic interaction between the ornithine of the

aminolipid and the protein of the surface of the erythrocytes. In addition, the hemagglutinating activity of phosphatidylserine was due to its similar structure to that of the ornithine-containing lipid and the mechanism was also presumed to be similar. The mechanism of hemagglutination by these aminolipids was distinct from that of lectins.

L18 ANSWER 25 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1982:215399 HCAPLUS
DOCUMENT NUMBER: 96:215399
TITLE: Detection, isolation, and analysis of a released **Bordetella pertussis product** toxic to cultured tracheal cells
AUTHOR(S): Goldman, William E.; Klapper, David G.; Baseman, Joel B.
CORPORATE SOURCE: Sch. Med., Univ. North Carolina, Chapel Hill, NC, 27514, USA
SOURCE: Infection and Immunity (1982), 36(2), 782-94
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Cultured hamster trachea epithelial cells were selected as an in vitro model system to study B. pertussis pathogenesis in the respiratory tract. DNA **synthesis** by serum-stimulated tracheal cells, in contrast to other cell types tested, was inhibited by the supernatant from log-phase B. pertussis broth cultures. A microassay with these tracheal cells permitted the development of a chromatog. purification scheme based on aggregation of the biol. activity under salt-free conditions. The active fraction from the 1st stage of purification caused a dose-dependent inhibition of DNA **synthesis** without a similar effect on RNA or protein **synthesis.** Organ cultures of hamster tracheal rings, when exposed to this partially purified fraction, developed epithelial cytopathol. comparable to that seen during B. pertussis infection. Ciliary activity slowed and eventually ceased as ciliated cells were extruded from the ring, leaving an intact but mostly nonciliated epithelium. Further purification of this biol. activity was achieved with **preparative** -scale high-voltage paper electrophoresis. Based on ninhydrin staining and the radioactive profile of material purified from radiolabeled B. pertussis cultures, 4 fractions were eluted from the paper by descending chromatog. Only component B caused a dose-dependent inhibition of cultured trachea cell DNA **synthesis** and epithelial cytopathol. in tracheal rings. Combination expts. also demonstrated enhanced inhibition by component B in the presence of component G (oxidized GSH), a copurifying mol. from the growth medium. Amino acid anal. of component B revealed a composition of glutamic acid (5 residues), alanine (5 residues), glycine (2 residues), **cysteine** (2 residues), and diaminopimelic acid (1 residue), as well as muramic acid and glucosamine.

L18 ANSWER 26 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1960:45774 HCAPLUS
DOCUMENT NUMBER: 54:45774
ORIGINAL REFERENCE NO.: 54:9063a-b
TITLE: Relation between pertussis toxin and mouse protection. I. Identification of Number 33 culture **Bordetella pertussis** and **production** of the toxin
AUTHOR(S): Naka, Keishiro
CORPORATE SOURCE: Osaka City Univ. Med. School
SOURCE: Nippon Saikingaku Zasshi (1958), 13, 947-54
CODEN: NSKZAM; ISSN: 0021-4930
DOCUMENT TYPE: Journal

LANGUAGE: Unavailable
AB The best medium for the toxin **production** was 1 l. extract of bovine heart, containing 20 g. polypeptone, 0.01 nicotinic acid, 0.2 **cysteine** hydrochloride, 5.0 NaCl, 0.4 MgCl2, and 1.0 KH2PO4. The toxin was purified and submitted to zone electrophoresis with starch as carrier. The thermolabile toxin fraction and the agglutinogen moved toward the anode but with different speeds; both appeared to be proteins. Dialysis of the toxin fraction caused complete loss of the toxicity.

L18 ANSWER 27 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1958:52768 HCAPLUS
DOCUMENT NUMBER: 52:52768
ORIGINAL REFERENCE NO.: 52:9528f-g
TITLE: Effective agent for pertussis
INVENTOR(S): Kuwashima, Kaneo
DOCUMENT TYPE: Patent
LANGUAGE: Unavailable
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 31008600		19561005	JP	

AB The hydrolyzed **product** of casein (10.0 g.), 2.5 g. NaCl, 0.5 g. K2HPO4, 11.0 cc. 1% CaCl2, 2.0 cc. 0.5% FeCl3, 1.0 cc. 0.1% MnCl2, 1.0 cc. 0.1% ZnCl2, 1.0 cc. 0.05% CuSO4, 10-4M vitamin B1, and 0.5 g. **cysteine**-HCl were dissolved in 1000 cc. H2O, the pH adjusted to 7.0, and 5-6 glass particles of 6-8 mm. in diameter added. The composition was sterilized at 121° and 15 lb./sq. in. for 30 min., inoculated with freshly cultured pertussis bacteria, kept at 37° for 48 hrs., and filtered.

L18 ANSWER 28 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1956:16771 HCAPLUS
DOCUMENT NUMBER: 50:16771
ORIGINAL REFERENCE NO.: 50:3545e-f
TITLE: **Synthetic** media for Hemophilus pertussis. III
AUTHOR(S): Arai, Shunichi
CORPORATE SOURCE: Maebashi Med. Coll., Gumma-ken
SOURCE: Japan. J. Bacteriol. (1952), 7, 557-60
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB A medium consisting of the 3 amino acids constituting glutathione permitted good growth of H. pertussis. Addition of histidine or tyrosine inhibited growth considerably while aspartic acid, asparagine, or arginine inhibited slightly; leucine had no effect. Addition of glycine to the medium containing **cysteine** and glutamic acid increased the growth greatly; asparagine and aspartic acid also increased growth. Tyrosine and leucine inhibited growth. Histidine and arginine had no effect. The medium containing aspartic acid and **cysteine** was augmented in the growth-stimulating activity by the addition of glutamic acid, asparagine, or arginine and was decreased in activity by addition of leucine, tyrosine, or glycine; histidine had no effect. The significance of individual amino acids in the nutrition of H. pertussis was discussed.

L18 ANSWER 29 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1956:16770 HCAPLUS
DOCUMENT NUMBER: 50:16770
ORIGINAL REFERENCE NO.: 50:3545c-e

TITLE: **Synthetic** media for Hemophilus pertussis. II
AUTHOR(S): Arai, Shunichi
CORPORATE SOURCE: Maebashi Med. Coll., Gumma-ken
SOURCE: Japan. J. Bacteriol. (1952), 7, 481-4
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. C.A. 49, 10424a, 11072c. Hemophilus pertussis grew slightly on **cysteine** alone, but addition in decreasing order of asparagine, aspartic acid, and glutamic acid improved growth considerably; addition of tyrosine or glycine gave no growth. Arginine, histidine, and leucine had no influence. The bacteria grew well on a medium containing all of these amino acids. The medium containing **cysteine**, glutamic acid, and glycine **produced** considerable growth. In a medium containing only asparagine addition of **cysteine** gave good growth, but arginine or glycine **produced** only slight growth; glutamic acid, leucine, histidine, aspartic acid, and tyrosine gave no growth. Combination of 2 amino acids among tyrosine, histidine, glycine, and leucine or addition of **cysteine** to each pair allowed no growth. The significance of a balanced combination of amino acids was discussed.

L18 ANSWER 30 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1956:16765 HCAPLUS
DOCUMENT NUMBER: 50:16765
ORIGINAL REFERENCE NO.: 50:3544b-e
TITLE: The fluid media of Hemophilus pertussis. I. Nutritional requirements of phase-1 Hemophilus pertussis
AUTHOR(S): Asano, Asao
CORPORATE SOURCE: Osaka City Med. School
SOURCE: Japan. J. Bacteriol. (1954), 9, 279-82
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. C.A. 48, 13809g. The liquid media for H. pertussis hitherto reported, which contain C powder or soluble starch are not convenient for the study of soluble antigens or toxins due to the action of these adsorbents. Addition of glass beads to the medium gave vigorous growth of the phase 1 H. pertussis in the absence of such adsorbents. The min. medium for the S type H. pertussis (Masui, et al., Osaka City Med. J. 1954, 1), consisting of 1% glutamic acid, 0.05% **cysteine**-HCl, and 10 γ/ml. nicotinic acid, did not support growth of phase 1 H. pertussis on static culture but did to some degree on shake culture. Addition of glass beads particularly in shake culture allowed vigorous growth, indicating under these conditions, the nutritional requirements of phase 1 H. pertussis to be the same as that of the S type. Nicotinic acid as the growth factor could not be replaced by other vitamins. Pyridoxine, pantothenic acid, and vitamin B12 were synergetic with nicotinic acid, suggesting that these vitamins were slowly **synthesized** by H. pertussis. Thiamine, riboflavine, p-aminobenzoic acid, folic acid, biotin, choline, adenosine, uracil, adenylic acid, and glutamine were not synergetic. (NH4)2SO4 did not replace glutamic acid as N source. On the basal medium addns. of tryptophan, arginine, and proline did not stimulate growth. No increase of the growth was observed by the addns. of glucose, glycerol, citric acid, malonic acid, fumaric acid, AcOH, lactic acid, succinic acid, maleic acid, pyruvic acid, and α-ketoglutaric acid; permeability of cell walls of H. pertussis may be a limiting factor for no utilization of these. Sulfate, SO3--, and S2O3-- were not utilized as a S source; the only S sources suitable were **cysteine** and methionine.

L18 ANSWER 31 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1955:53969 HCAPLUS

DOCUMENT NUMBER: 49:53969
ORIGINAL REFERENCE NO.: 49:10424a-b
TITLE: **Synthetic** media for Hemophilus pertussis. IV. The concentrations of several amino acids. 1
AUTHOR(S): Arai, Shunichi
CORPORATE SOURCE: Maebashi Med. Coll., Gumma-ken
SOURCE: Japan. J. Bacteriol. (1953), 8, 13-15
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. C.A. 48, 12283h. In a medium in which **cysteine** was the sole N source, bacterial growth was only slightly affected by concentration When **cysteine**, glutamic acid, and glycine were added together, variations in the concentration of **cysteine** influenced growth considerably. In both cases, concns. of amino acids lower than a definite level decreased growth. When glutamic acid was added alone, a high concentration did not **produce** bacterial growth, but in the presence of **cysteine** and glycine, growth was directly related to concentration Glycine, alone or in combination with 2 other amino acids, had slight affect. Of the three amino acids glutamic acid was the most important in **synthesis** of bacterial proteins.

L18 ANSWER 32 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1955:24458 HCAPLUS
DOCUMENT NUMBER: 49:24458
ORIGINAL REFERENCE NO.: 49:4782b-c
TITLE: Metabolism of Hemophilus pertussis. I. The metabolic cycle
AUTHOR(S): Abe, Teitaro
CORPORATE SOURCE: Natl. Inst.
SOURCE: Japanese Journal of Experimental Medicine (1953), 23, 197-203
CODEN: JJEMAG; ISSN: 0021-5031
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB Oxygen consumption of resting cells of Hemophilus pertussis was determined with various substrates by using the Warburg respirometer. Among the sugars tested, none of the disaccharides, hexoses, pentoses, or sugar alcs. was oxidized except glucosamine. Most amino acids were oxidized except norleucine, histidine, β-alanine, aminobutyric acid, methionine, and taurine. Citric acid cycle intermediates were metabolized, although the rate was slower than that observed with Escherichia coli. Growth expts. were carried out with certain substrates in a **synthetic** liquid medium. Glutamic acid was found to support good growth and **cysteine**, methionine, glutathione as well as Na2S served as favorable S sources.

L18 ANSWER 33 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1954:78332 HCAPLUS
DOCUMENT NUMBER: 48:78332
ORIGINAL REFERENCE NO.: 48:13822c-e
TITLE: Investigation of amino acid metabolism in Hemophilus pertussis by paper chromatography
AUTHOR(S): Miyamoto, Haruo; Akama, Kiyoto; Morita, Etsuko; Ikeda, Toshio
CORPORATE SOURCE: Gunma Univ., Maebashi, Japan
SOURCE: Gunma Journal of Medical Sciences (1953), 2, 157-62
CODEN: GJMSA7; ISSN: 0017-565X
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB cf. Arai, Nihon Saikingaku Zasshi. 8, 175(1953). Amino acid metabolism was studied in H. pertussis cultured on a **synthetic** medium, which contained as the N source 0.02 g./l. L-**cysteine**-HCl, 0.2 g./l. D-glutamic acid, and 0.02 g./l. glycine, by measuring the disappearance of added amino acids. When glutamic acid concentration was increased to 2.5 g./l., 75% disappeared in 8 days; when the **cysteine** concentration was increased to 1.5 g./l., 87.5% disappeared; when the glycine concentration was increased to 1.25 g./l., 50% disappeared; when 1 g./l. L-tyrosine was added, 75% disappeared; when 5 g./l. DL-asparagine was added, 50% disappeared; when 1 g./l. DL-aspartic acid was added, 50% disappeared; when 2.5 g./l. L-histidine-HCl was added, 50% disappeared; when 3 g./l. L-arginine-HCl was added, 50% disappeared, and when 1.25 g./l. L-leucine was added, 50% disappeared.

L18 ANSWER 34 OF 34 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1954:60868 HCAPLUS
DOCUMENT NUMBER: 48:60868
ORIGINAL REFERENCE NO.: 48:10832a-c
TITLE: Metabolism of Hemophilus pertussis. II. The metabolic mode
AUTHOR(S): Abe, Sadataro
CORPORATE SOURCE: Natl. Inst. Health, Japan, Tokyo
SOURCE: Japan J. Bacteriol. (1952), 7, 81-4
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB cf. C.A. 48, 10115d. As determined by the Warburg manometer, H. pertussis on Bordet-Gengou medium did not utilize sugars and sugar alcs. as an energy source; glucosamine was utilized to a small extent. The following amino acids were utilized (with Qo2): L-glutamic acid 347, DL-serine 67, aspartic acid 152, L-proline 162, hydroxyproline 90, L-leucine 47, glycine 38, lysine 80, arginine 67, phenylalanine 38, **cysteine** 423, tryptophan 47, DL-alanine 128, valine 38, norvaline 128. A **synthetic** medium is described. Pyridoxine stimulated growth but was not indispensable. Of S sources, glutathione was best followed by **cysteine** and methionine. The Qo2 of sugar-metabolism intermediates were: succinic acid 298, fumaric acid 83, malic acid 41, pyruvic acid 36, α-ketoglutaric acid 127, oxalacetic acid 10, citric acid 75, acetic acid 16, glycerol 7, and oxalic, malonic, tartaric, and formic acids 0. The enzyme system of sugar metabolism in H. pertussis seems to be inferior to that in Escherichia coli. It is indicated that the energy sources are introduced via oxalacetic acid and α-ketoglutaric acid into a Krebs cycle; the route from carbohydrates via pyruvic acid is excluded.

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=> d que stat l20
L13             1 SEA FILE=REGISTRY ABB=ON  "BORDETELLA PERTUSSIS TOXIN"/CN
L14             2 SEA FILE=REGISTRY ABB=ON  CYSTEINE/CN
L15            50 SEA FILE=HCAPLUS ABB=ON   (L13 OR ?BORDETELLA?(W)?PERTUSSIS?)
                  AND (L14 OR ?CYSTEINE?)
L16            34 SEA FILE=HCAPLUS ABB=ON   L15 AND (?PRODUC? OR ?MANUF? OR
                  ?PREP? OR ?SYNTH?)
L17            15 SEA FILE=HCAPLUS ABB=ON   L16 AND (?METHOD? OR ?TECHNIQ? OR
                  ?PROCED? OR ?PROCES?)
L19            18 SEA L17
L20            14 DUP REMOV L19 (4 DUPLICATES REMOVED)

=> d ibib abs l20 1-14

L20  ANSWER 1 OF 14     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:    2003084092     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 12595447
TITLE:               Reduced glutathione is required for pertussis toxin
                     secretion by Bordetella pertussis.
AUTHOR:              Stenson Trevor H; Patton Angela K; Weiss Alison A
CORPORATE SOURCE:    Department of Molecular Genetics, Biochemistry, and
                     Microbiology, University of Cincinnati, Cincinnati, Ohio
                     45267-0524, USA.
CONTRACT NUMBER:     R01 AI23695 (NIAID)
SOURCE:              Infection and immunity, (2003 Mar) 71 (3) 1316-20.
                     Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         200303
ENTRY DATE:          Entered STN: 20030222
                     Last Updated on STN: 20030321
                     Entered Medline: 20030320
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AB The abilities of **cysteine**-containing compounds to support growth of **Bordetella pertussis** and influence pertussis toxin transcription, assembly, and secretion were examined. **Cysteine** is an essential amino acid for B. pertussis and must be present for protein **synthesis** and bacterial growth. However, **cysteine** can be metabolized to sulfate, and high concentrations of sulfate can selectively inhibit transcription of the virulence factors, including pertussis toxin, via the BvgAS two-component regulatory system in a **process** called modulation. In addition, pertussis toxin possesses several disulfide bonds, and the **cysteine**-containing compound glutathione can influence oxidation-reduction reactions and perhaps disulfide bond formation. Bacterial growth was not observed in the absence of a source of **cysteine.** Oxidized glutathione, as a sole source of **cysteine**, also did not support bacterial growth. **Cysteine**, cystine, and reduced glutathione did support bacterial growth, and none of these compounds caused modulation at the concentrations tested. Similar amounts of periplasmic pertussis toxin were detected regardless of the source of **cysteine**; however, in the absence of reduced glutathione, pertussis toxin was not efficiently secreted. Addition of the reducing agent dithiothreitol was unable to compensate for the lack of reduced glutathione and did not promote secretion of pertussis toxin. These results suggest that reduced glutathione does not affect the accumulation of assembled active pertussis toxin in the periplasm but plays a role in efficient pertussis toxin secretion by the bacterium.

L20 ANSWER 2 OF 14 WPIDS COPYRIGHT 2004 THE THOMSON CORP on STN
ACCESSION NUMBER: 2002-010777 [01] WPIDS
DOC. NO. CPI: C2002-002634
TITLE: Enhancing **production** of bacterial toxins comprises eliminating or reducing toxin expression inhibitors formed by toxin **producing** bacteria by adding at least one soluble metal salt that forms an insoluble complex with sulfate.
DERWENT CLASS: B04 D16
INVENTOR(S): BLAKE, M S; BOGDAN, J A; NAZARIO-LARRIEU, J
PATENT ASSIGNEE(S): (BAXT) BAXTER HEALTHCARE SA; (BAXT) BAXTER INT INC; (BLAK-I) BLAKE M S; (BOGD-I) BOGDAN J A; (NAZA-I) NAZARIO-LARRIEU J
COUNTRY COUNT: 96
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001074862	A2	20011011	(200201)*	EN	46
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2001053134	A	20011015	(200209)		
US 2002061555	A1	20020523	(200239)		
US 2002165344	A1	20021107	(200275)		
EP 1268531	A2	20030102	(200310)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					
JP 2003531586	W	20031028	(200373)		54
US 6686180	B2	20040203	(200413)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001074862	A2	WO 2001-US10938	20010404
AU 2001053134	A	AU 2001-53134	20010404
US 2002061555	A1 Provisional	US 2000-194482P	20000404
		US 2001-825770	20010404
US 2002165344	A1 Provisional	US 2000-194478P	20000404
		US 2001-825769	20010404
EP 1268531	A2	EP 2001-926612	20010404
		WO 2001-US10938	20010404
JP 2003531586	W	JP 2001-572551	20010404
		WO 2001-US10938	20010404
US 6686180	B2 Provisional	US 2000-194482P	20000404
		US 2001-825770	20010404

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001053134	A	Based on	WO 2001074862
EP 1268531	A2	Based on	WO 2001074862
JP 2003531586	W	Based on	WO 2001074862

PRIORITY APPLN. INFO: US 2000-194482P 20000404; US

2000-194478P 20000404; US
2001-825770 20010404; US
2001-825769 20010404

AN 2002-010777 [01] WPIDS
AB WO 200174862 A UPAB: 20040210
NOVELTY - Enhancing **production** of bacterial toxins comprises eliminating or reducing toxin expression inhibitors formed by toxin **producing** bacteria.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a **method** of cultivating **Bordetella pertussis** in the presence of one or more soluble metal salts that form a substantially insoluble complex with sulfate;

(2) a **method** of making a culture medium that supports B. pertussis growth and prevents or decreases inhibition of pertussis toxin (PT) expression by sulfate, by admixing a B. pertussis culture medium with one or more soluble metal salts that form a substantially insoluble complex with sulfate;

(3) a culture medium that supports the growth of B. pertussis comprising one or more soluble metal salts that form a substantially insoluble complex with sulfate, where the amount prevents or reduces the inhibition of PT expression by sulfate;

(4) **methods** of **producing** PT comprising growing B. pertussis in a medium comprising a soluble metal salt that forms an insoluble complex with sulfate, and isolating the PT from the culture medium;

(5) a B. pertussis **cysteine** desulfinase knockout mutant;

(6) a **method** of enhanced **production** of PT by cultivating B. pertussis **cysteine** desulfinase knockout mutant where an enhanced amount of PT **produced** is compared to when a non-**cysteine** desulfinase knockout mutant is employed;

(7) a peptide comprising the amino acid sequence GGGDGSFSGFGDGSFSGFG-OH (I);

(8) a **method** of isolating a bacterial toxin from a mixture by **preparing** a peptide affinity column where the peptide is (I), comprising:

(a) adding the mixture containing the bacterial toxin to the peptide affinity column, where the bacterial toxin binds to the peptide;

(b) releasing the bound bacterial toxin from the peptide; and

(c) collecting the isolated bacterial toxin.

USE - The **method** is useful for increasing **production** of pertussis toxin by reducing or eliminating the accumulation of Bordetella species toxin expression inhibitors.

ADVANTAGE - Compared with previous **methods** of **producing** PT, e.g. growing B. pertussis in a stationary culture which is labor intensive, or cultivation on a fermentation scale which requires vortex stirring and surface modification, the new **method** results to increased or higher **production** of toxins.

Dwg.0/8

L20 ANSWER 3 OF 14 WPIDS COPYRIGHT 2004 THE THOMSON CORP on STN
ACCESSION NUMBER: 2001-226496 [23] WPIDS
DOC. NO. CPI: C2001-067567
TITLE: An isolated compound for inhibiting pilus assembly.
DERWENT CLASS: B04 C03
INVENTOR(S): FUETTERER, K; HULTGREN, S J; SAUER, F G; WAKSMAN, G
PATENT ASSIGNEE(S): (UNIW) UNIV WASHINGTON
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001010386	A2	20010215	(200123)*	EN	142
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2000074703	A	20010305	(200130)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001010386	A2	WO 2000-US22087	20000811
AU 2000074703	A	AU 2000-74703	20000811

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000074703	A	Based on	WO 2001010386

PRIORITY APPLN. INFO: US 1999-148280P 19990811

AN 2001-226496 [23] WPIDS

AB WO 200110386 A UPAB: 20010425

NOVELTY - An isolated compound (I) which binds to a pilius subunit groove, and hence inhibits pilus assembly, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a mannose analog (II) capable of competitively binding the amino terminal mannose-binding domain of a Gram-negative adhesin (III);

(2) a composition (C) comprising a pilus chaperone-subunit co-complex (IV) in crystalline form, where the co-complex contains an amino acid sequence of a G1 beta-strand of a chaperone and an amino-terminal end of a pilus subunit;

(3) **producing** (M1) a PapD-PapK chaperone-subunit co-complex (V) in crystalline form, comprising:

(a) mixing a solution comprising (V) with a reservoir solution containing a precipitant; and

(b) incubating the mixture obtained in (a) over the reservoir solution in a closed container, under conditions suitable for crystallisation until the crystal forms;

(4) identifying (M2) an antibacterial compound, using a three-dimensional (3D) structural representation of (IV) (or a fragment of (IV)) containing a G1 beta-strand binding cleft, to computationally screen a candidate compound for an ability to bind the beta-strand binding cleft;

(5) identifying (M3) an antibacterial compound, using a 3D structural representation of (IV) (or a fragment of (IV)), comprising a G1 beta-strand binding cleft, to computationally design a **synthesizable** compound that binds the G1 beta-strand binding cleft;

(6) identifying (M4) an antibacterial compound, using a 3D structural representation of a chaperone (VI) (or a fragment of (VI)), comprising a G1 beta-strand, to identify or design a compound with a 3D structure similar to the 3D structure of the G1 beta-strand of (VI);

(7) identifying (M5) an antibacterial compound, using a 3D structural representation of an adhesin (VII) (or a fragment of (VII)), comprising a lectin binding domain, to screen a candidate compound for the ability to

bind a lectin binding domain of (VII); and

(8) identifying (M6) an antibacterial compound, using a 3D structural representation of an adhesin (VII) (or a fragment of (VII)), comprising a lectin binding domain, to computationally design a compound that binds the lectin binding domain of (VII);

(9) a machine-readable medium embedded with information that corresponds to the 3D structural representation of (IV).

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Periplasmic chaperone to pilus subunit inhibitor; pilus formation inhibitor.

No details given.

USE - The compound (I) is used in a **method** for preventing or inhibiting:

(a) the formation of pilus subunit-subunit structure;

(b) the formation of a chaperone-subunit structure;

(c) pili adhesion to a host tissue, comprising the administration of a mannose analogue;

(d) inhibiting bacterial colonization by a Gram-negative organism

Compound (I) and (II) exhibit antibacterial activity against Escherichia coli, Haemophilus influenzae, H. influenzae,, Salmonella enteriditis, S. tyhimurium, **Bordetella pertussis**, Yersinia pestis, Y. enterocolitica, Helicobacter pylori and Klebsiella pneumoniae.

The antibacterial inhibition activity is useful for treating mammals (including humans) and plants (all claimed).

Dwg.0/32

L20 ANSWER 4 OF 14 MEDLINE on STN DUPLICATE 2

ACCESSION NUMBER: 2001533919 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11580213
TITLE: A quantitative analysis for the ADP-ribosylation activity of pertussis toxin: an enzymatic-HPLC coupled assay applicable to formulated whole cell and acellular pertussis vaccine **products.**
AUTHOR: Cyr T; Menzies A J; Calver J; Whitehouse L W
CORPORATE SOURCE: Research Services Division, Bureau of Biologics and Radiopharmaceuticals, Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Tunney's Pasture, Ottawa, K1A 0L2, Canada.. terry_cyr@hc-sc.gc.ca
SOURCE: Biologicals : journal of the International Association of Biological Standardization, (2001 Jun) 29 (2) 81-95. Journal code: 9004494. ISSN: 1045-1056.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 20011003
Last Updated on STN: 20021218
Entered Medline: 20020122

AB The majority of the biological effects of pertussis toxin (PT) are the result of a toxin-catalyzed transfer of an adenosine diphosphate-ribose (ADP-ribose) moiety from NAD(+)to the alpha-subunits of a subset of signal-transducing guanine-nucleotide-binding proteins (G-proteins). This generally leads to an uncoupling of the modified G-protein from the corresponding receptor and the loss of effector regulation. This assay is based on the PT S1 subunit enzymatic transfer of ADP-ribose from NAD to the **cysteine** moiety of a fluorescent tagged **synthetic** peptide homologous to the 20 amino acid residue carboxyl-terminal sequence

of the alpha-subunit of the G(i3)protein. The tagged peptide and the ADP-ribosylated **product** were characterized by HPLC/MS and MS/MS for structure confirmation. Quantitation of this characterized ADP-ribosylated fluorescently tagged peptide was by HPLC fluorescence using Standard Addition **methodology.** The assay was linear over a five hr incubation period at 20 degrees C at PT concentrations between 0.0625 and 4.0 microg/ml and the sensitivity of the assay could be increased several fold by increasing the incubation time to 24 h. Purified S1 subunit of PT exhibited 68.1+/-10.1% of the activity of the intact toxin on a molar basis, whereas the pertussis toxin B oligomer, the genetically engineered toxoid, (PT-9K/129G), and several of the other components of the **Bordetella pertussis** organism possessed little (<0.6%) or no detectable ribosylation activity. Commonly used pertussis vaccine reference materials, US PV Lot #11, BRP PV 66/303, and BRP PV 88/522, were assayed by this **method** against **Bordetella pertussis** Toxin Standard 90/518 and demonstrated to contain, respectively, 0.323+/-0.007, 0.682+/-0.045, and 0.757+/-0.006 microg PT/ml (Mean+/-SEM) or in terms of microg/vial: 3.63, 4.09 and 4.54, respectively. A survey of several multivalent pertussis vaccine **products** formulated with both whole cell as well as acellular components indicated that **products** possessed a wide range of ribosylation activities. The pertussis toxin S1 subunit catalyzed ADP- ribosylation of the FAC-Galpha(i3)C20 peptide substrate and its subsequent quantitation by HPLC was demonstrated to be a sensitive and quantitative **method** for measuring intrinsic pertussis toxin activity. This **methodology** not only has the potential to be an alternative physicochemical **method** to replace existing bioassay **methodology**, but has the added advantage of being a universal **method** applicable to the assay of pertussis toxin in both whole cell and acellular vaccines as well as bulk and final formulated vaccine **products.** Acceptance of this **method** by regulatory agencies and industry as a credible alternative to existing **methods** would, however, require validation in an international collaborative study against the widely accepted bioassay **methods**

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L20 ANSWER 5 OF 14 MEDLINE on STN
ACCESSION NUMBER: 2001193593 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11207619
TITLE: Expression, activity and cytotoxicity of pertussis toxin S1 subunit in transfected mammalian cells.
AUTHOR: Castro M G; McNamara U; Carbonetti N H
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore 21201, USA.
CONTRACT NUMBER: AI38979 (NIAID)
AI42681 (NIAID)
SOURCE: Cellular microbiology, (2001 Jan) 3 (1) 45-54.
Journal code: 100883691. ISSN: 1462-5814.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010410
Last Updated on STN: 20021218
Entered Medline: 20010405

AB Pertussis toxin (PT) comprises an active subunit (S1), which ADP-ribosylates the alpha subunit of several mammalian G proteins, and the B oligomer (S2-S5), which binds glycoconjugate receptors on cells. In a

previous report, expression of S1 in Cos cells resulted in no observable cytotoxicity, and it was hypothesized that either S1 failed to locate its target proteins or the B oligomer was also necessary for cytotoxicity. To address this, we stably transfected S1 with and without a signal peptide into mammalian cells. Immunofluorescence analysis confirmed the function of the signal peptide. Surprisingly, we found that S1 was active in both transfectants, as determined by clustering of transfected Chinese hamster ovary (CHO) cells and ADP-ribosylation of G proteins. Constructs with a **cysteine**-to-serine change at residue 201 or a truncated S1 (residues 1-181) were also active when transfected into cells. Constructs with an inactive mutant S1 had no activity, confirming that the observed results were due to the activity of the toxin subunit. We conclude that S1 is active when expressed in mammalian cells without the B oligomer, that secretion into the endoplasmic reticulum does not prevent this activity and that the C-terminal portion of S1 is not required for its activity in cells.

L20 ANSWER 6 OF 14 WPIDS COPYRIGHT 2004 THE THOMSON CORP on STN
ACCESSION NUMBER: 2001-032017 [04] WPIDS
DOC. NO. CPI: C2001-009841
TITLE: Novel fluorescent proteins comprising a sensor protein inserted into them, useful for measuring the response of a sensor biological, chemical, electrical or physiological parameter in vivo or in vitro.
DERWENT CLASS: B04 D16
INVENTOR(S): BAIRD, G A; TSIEN, R Y
PATENT ASSIGNEE(S): (REGC) UNIV CALIFORNIA; (BAIR-I) BAIRD G; (TSIE-I) TSIEN R Y
COUNTRY COUNT: 92
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000071565	A2	20001130	(200104)*	EN	94
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2000052751	A	20001212	(200115)		
US 6469154	B1	20021022	(200273)		
US 2002157120	A1	20021024	(200273)		
US 6699687	B1	20040302	(200417)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000071565	A2	WO 2000-US13684	20000517
AU 2000052751	A	AU 2000-52751	20000517
US 6469154	B1	US 1999-316919	19990521
US 2002157120	A1 CIP of	US 1999-316920	19990521
		US 2001-999745	20011023
US 6699687	B1	US 1999-316920	19990521

FILING DETAILS:

PATENT NO	KIND	PATENT NO

AU 2000052751 A Based on WO 2000071565

PRIORITY APPLN. INFO: US 1999-316920 19990521; US 1999-316919 19990521

AN 2001-032017 [04] WPIDS

AB WO 200071565 A UPAB: 20010118

NOVELTY - An isolated polypeptide (I) comprising a fluorescent indicator (FI) which comprises a sensor polypeptide (II) that is responsive to a chemical, biological, electrical or physiological parameter, and a fluorescence protein functional group (III), is new. (II) is operatively inserted into (III), and the fluorescence of (III) is affected by the responsiveness of (II).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated nucleic acid sequence (IV) which encodes a FI which comprises a (II) and (III);

(2) an expression vector (V) containing (IV);

(3) a transgenic non-human animal comprising (IV);

(4) an expression vector (VI) comprising expression control sequences operatively linked to (IV);

(5) a host cell (VII) transfected with (VI);

(6) FI which comprises (II) and (III);

(7) an isolated nucleic acid sequence (VIII) encoding a circularly permuted fluorescent protein moiety (IX) which comprises a linker group (LM) linking the amino-terminal and carboxy-terminal amino acids of a fluorescent protein, in which the amino and carboxy termini are linked as internal amino acids in the (IX) and two terminal ends (TTE) in which the first end is an amino-terminal end and the second end is a carboxy terminal end and in which the amino and carboxy terminal ends of (IX) are different from the amino-terminal and carboxy-terminal amino acids of the fluorescent protein;

(8) an expression vector and transgenic non-human animal comprising (VIII);

(9) an expression vector (X) comprising expression control sequences operatively linked to (VIII);

(10) a host cell (XI) transfected with (X);

(11) an isolated polypeptide comprising (IX) which comprises LM, TTE and also (II);

(12) **producing** (VIII) involves linking a nucleic sequence LM to the 5' nucleotide of a polynucleotide encoding a fluorescent protein, circularizing the polynucleotide with the nucleic acid sequence encoding the linker sequence and cleaving the circularized polynucleotide with a nuclease, by which cleavage the circularized polynucleotide is linearized; and

(13) **producing** (IX) involves expressing the nucleic acid **produced** by the **method** in (12).

USE - FI is useful for detecting the presence of a response inducing member in a sample. The **method** involves contacting the sample with FI and detecting a change in fluorescence, in which a change is indicative of the affect (a change in electrical or chemical potential) of the parameter on the sensor polypeptide (claimed). (I) or (IX) is also useful for determining the presence of a chemical, biological, electrical or physiological parameter and thus is useful for determining if a cell exhibits an activity, which involves transfecting the cell with a nucleic acid encoding FI or (IX), exciting FI or (IX) and measuring the amount of an optical property in the presence and absence of the activity, such that a change in the optical property is indicative of activity and also for determining transient changes in a chemical, biological, electrical or physiological parameter which involves contacting a cell with FI or (IX) and measuring a change in the optical property of the indicator over time.

ADVANTAGE - The novel fluorescent proteins are advantageous due their reduced size as compared to the FRET (fluorescence resonance energy transfer)-based sensors. The reduced size has importance in allowing the indicator to measure chemical, biological, electrical or physiological interactions with the sensor polypeptide in, e.g. subcellular compartments previously inaccessible to the larger, FRET-based sensors. In addition, the maximal change in fluorescence intensity observed in the present indicators (e.g. up to 8 fold increase) are much larger than those in the cameleons (e.g. FRET-based sensors), which show only a 2 fold change in yellow to cyan intensity ratio.

DESCRIPTION OF DRAWING(S) - The figure shows the overall design of a circularly permutated polypeptide.

Dwg.5/8

L20 ANSWER 7 OF 14 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1999:235929 BIOSIS
DOCUMENT NUMBER: PREV199900235929
TITLE: The conserved lysine 860 in the additional fatty-acylation site of **Bordetella pertussis** adenylate cyclase is crucial for toxin function independently of its acylation status.
AUTHOR(S): Basar, Tumay; Havlicek, Vladimir; Bezouskova, Silvia; Halada, Petr; Hackett, Murray; Sebo, Peter [Reprint author]
CORPORATE SOURCE: Institute of Microbiology CAS, Videnska 1083, CZ-142 20, Prague 4, Czech Republic
SOURCE: Journal of Biological Chemistry, (April 16, 1999) Vol. 274, No. 16, pp. 10777-10783. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 17 Jun 1999
Last Updated on STN: 17 Jun 1999

AB The **Bordetella pertussis** RTX (repeat in toxin family protein) adenylate cyclase toxin-hemolysin (ACT) acquires biological activity upon a single amide-linked palmitoylation of the epsilon-amino group of lysine 983 (Lys983) by the accessory fatty-acyltransferase CyaC. However, an additional conserved RTX acylation site can be identified in ACT at lysine 860 (Lys860), and this residue becomes palmitoylated when recombinant ACT (r-Ec-ACT) is **produced** together with CyaC in Escherichia coli K12. We have eliminated this additional acylation site by replacing Lys860 of ACT with arginine, leucine, and **cysteine** residues. Two-dimensional gel electrophoresis and microcapillary high performance liquid chromatography/tandem mass spectrometric analyses of mutant proteins confirmed that the two sites are acylated independently in vivo and that mutations of Lys860 did not affect the quantitative acylation of Lys983 by palmitoyl (C16:0) and palmitoleil (cis DELTA9 C16:1) fatty-acyl groups. Nevertheless, even the most conservative substitution of lysine 860 by an arginine residue caused a 10-fold decrease of toxin activity. This resulted from a 5-fold reduction of cell association capacity and a further 2-fold reduction in cell penetration efficiency of the membrane-bound K860R toxin. These results suggest that lysine 860 plays by itself a crucial structural role in membrane insertion and translocation of the toxin, independently of its acylation status.

L20 ANSWER 8 OF 14 MEDLINE on STN

ACCESSION NUMBER: 1999138881 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9973458
TITLE: Direct delivery of the **Bordetella pertussis** adenylate cyclase toxin to the MHC class

I antigen presentation pathway.
AUTHOR: Guermonprez P; Ladant D; Karimova G; Ullmann A; Leclerc C
CORPORATE SOURCE: Unite de Biologie des Regulations Immunitaires, Institut Pasteur, Paris, France.
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (1999 Feb 15) 162 (4) 1910-6.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990426
Last Updated on STN: 20030110
Entered Medline: 19990413

AB Among bacterial toxins, the adenylate cyclase toxin of **Bordetella pertussis** (CyaA) has a unique mechanism of entry that consists in the direct translocation of its catalytic domain across the plasma membrane of target cell, a mechanism supposed to be independent of any endocytic pathway. Here, we report that the CyaA toxin is delivered to the cytosolic pathway for MHC class I-restricted Ag presentation. Using peritoneal macrophages as APC, we show that the OVA 257-264 CD8+ epitope genetically inserted into a detoxified CyaA (CyaA-OVA E5) is presented to CD8+ T cells by a mechanism requiring 1) proteasome **processing**, 2) TAP, and 3) **neosynthesis** of MHC class I. We demonstrate that the presentation of CyaA-OVA E5, like the translocation of CyaA into eukaryotic cells, is dependent on extracellular Ca2+ and independent of vacuolar acidification. Moreover, inhibitors of the phagocytic and macropinocytic endocytic pathways do not affect the CyaA-OVA E5 presentation. The absence of specific cellular receptors for CyaA correlates with the ability of various APC to present the recombinant CyaA toxin, including dendritic cells, macrophages, splenocytes, and lymphoid tumoral lines. Taken together, our results show that the CyaA presentation pathway is not cell type specific and is unrelated to a defined type of endocytic mechanism. Thus, it represents a new and unconventional delivery of an exogenous Ag into the conventional cytosolic pathway.

L20 ANSWER 9 OF 14 MEDLINE on STN
ACCESSION NUMBER: 1999115531 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9916065
TITLE: Intracellular delivery of a cytolytic T-lymphocyte epitope peptide by pertussis toxin to major histocompatibility complex class I without involvement of the cytosolic class I antigen **processing** pathway.
AUTHOR: Carbonetti N H; Irish T J; Chen C H; O'Connell C B; Hadley G A; McNamara U; Tuskan R G; Lewis G K
CORPORATE SOURCE: Departments of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA.. ncarbone@umaryland.edu
CONTRACT NUMBER: AI38192 (NIAID)
AI38979 (NIAID)
AI42681 (NIAID)
SOURCE: Infection and immunity, (1999 Feb) 67 (2) 602-7.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903

ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 20021218
Entered Medline: 19990309

AB A CD8(+) cytolytic T-lymphocyte (CTL) response to antigen-presenting cells generally requires intracellular delivery or **synthesis** of antigens in order to access the major histocompatibility complex (MHC) class I **processing** and presentation pathway. To test the ability of pertussis toxin (PT) to deliver peptides to the class I pathway for CTL recognition, we constructed fusions of CTL epitope peptides with a genetically detoxified derivative of PT (PT9K/129G). Two sites on the A (S1) subunit of PT9K/129G tolerated the insertion of peptides, allowing efficient assembly and secretion of the holotoxin fusion by **Bordetella pertussis.** Target cells incubated with these fusion proteins were specifically lysed by CTLs in vitro, and this activity was shown to be MHC class I restricted. The activity was inhibited by brefeldin A, suggesting a dependence on intracellular trafficking events, but was not inhibited by the proteasome inhibitors lactacystin and N-acetyl-L-leucyl-L-leucyl-L-norleucinal (LLnL). Furthermore, the activity was present in mutant antigen-presenting cells lacking the transporter associated with antigen **processing**, which transports peptides from the cytosol to the endoplasmic reticulum for association with MHC class I molecules. PT may therefore bypass the proteasome-dependent cytosolic pathway for antigen presentation and deliver epitopes to class I molecules via an alternative route.

L20 ANSWER 10 OF 14 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1998:352331 BIOSIS
DOCUMENT NUMBER: PREV199800352331
TITLE: N-terminal characterization of the **Bordetella pertussis** filamentous haemagglutinin.
AUTHOR(S): Lambert-Buisine, Corinne; Willery, Eve; Locht, Camille; Jacob-Dubuisson, Francoise [Reprint author]
CORPORATE SOURCE: Inst. U447, IBL, Inst. Pasteur de Lille, rue du Prof Calmette, 59019 Lille Cedex, France
SOURCE: Molecular Microbiology, (June, 1998) Vol. 28, No. 6, pp. 1283-1293. print.
CODEN: MOMIEE. ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Aug 1998
Last Updated on STN: 13 Aug 1998

AB The major adhesin of **Bordetella pertussis**, filamentous haemagglutinin (FHA), is **produced** and secreted at high levels by the bacterium. Mature FHA derives from a large precursor, FhaB, that undergoes several post-translational maturations. In this work, we demonstrate by site-directed mutagenesis that the N-terminal signal peptide of FHA Is composed of 71 amino acids, including a 22-residue-long 'N-terminal extension' sequence. This sequence, although highly conserved in various other secretory proteins, does not appear to play an essential part in FHA secretion, as shown by deletion mutagenesis. The entire N-terminal signal region of FhaB is removed in the course of secretion by proteolytic cleavage at a site that corresponds to a Lep signal peptidase recognition sequence. After this maturation, the N-terminal glutamine residue is modified to a pyroglutamate residue. This modification is not crucial for heparin binding, haemagglutination or secretion. Interestingly, however, the modification is absent from Escherichia coli secreted FHA derivatives. In addition, it is dependent in B. pertussis on the presence of all three **cysteines** contained in the signal peptide of FhaB. These observations suggest that it does not occur

spontaneously but perhaps requires a specific enzymatic machinery.

L20 ANSWER 11 OF 14 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 1998308142 EMBASE
TITLE: Inhibition of TGF-β-stimulated CTGF gene expression and anchorage- independent growth by cAMP identifies a CTGF-dependent restriction point in the cell cycle.
AUTHOR: Kothapalli D.; Hayashi N.; Grotendorst G.R.
CORPORATE SOURCE: G.R. Grotendorst, Dept. of Cell Biology and Anatomy, Univ. of Miami School of Medicine, 1600 N.W. 10th Ave., Miami, FL 33136, United States. ggrotend@mednet.med.miami.edu
SOURCE: FASEB Journal, (1998) 12/12 (1151-1161).
Refs: 34
ISSN: 0892-6638 CODEN: FAJOEC
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 013 Dermatology and Venereology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB CTGF is a 38 kDa **cysteine**-rich peptide whose **synthesis** and secretion are selectively induced by transforming growth factor β (TGF-β) in connective tissue cells. We have investigated the signaling pathways controlling the TGF-β induction of connective tissue growth factor (CTGF) gene expression. Our studies indicate that inhibitors of tyrosine kinases and protein kinase C do not block the signaling pathway used by TGF-β to induce CTGF gene expression. In contrast, elevation of cAMP levels within the target cells by a variety of **methods** blocked the induction of CTGF by TGF-β. Furthermore, agents that elevate cAMP blocked the induction of anchor-age-independent growth (AIG) by TGF-β. Inhibition of AIG could be overcome by the addition of CTGF, indicating that it was not a general inhibition of growth but a selective inhibition of CTGF **synthesis** that is responsible for the inhibition of TGF-β-induced AIG by cAMP. Kinetic studies of the induction of DNA **synthesis** by CTGF in cells arrested by cAMP indicate that the block occurs in very late G1. These and other studies in monolayer cultures suggest that the CTGF restriction point in the cell cycle is distinct from the adhesion- dependent arrest point.

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ACCESSION NUMBER: 94370912 EMBASE
DOCUMENT NUMBER: 1994370912
TITLE: Mechanisms of action of nitrates.
AUTHOR: Torfgard K.E.; Ahlner J.
CORPORATE SOURCE: Department of Clinical Pharmacology, University Hospital,S-581 85 Linkoping, Sweden
SOURCE: Cardiovascular Drugs and Therapy, (1994) 8/5 (701-717).
ISSN: 0920-3206 CODEN: CDTHET
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Glyceryl trinitrate, isosorbide dinitrate, and isosorbide-5-mononitrate are organic nitrate esters commonly used in the treatment of angina

pectoris, myocardial infarction, and congestive heart failure. Organic nitrate esters have a direct relaxant effect on vascular smooth muscles, and the dilation of coronary vessels improves oxygen supply to the myocardium. The dilation of peripheral veins, and in higher doses peripheral arteries, reduces preload and afterload, and thereby lowers myocardial oxygen consumption. Inhibition of platelet aggregation is another effect that is probably of therapeutic value. Effects on the central nervous system and the myocardium have been shown but not scrutinized for therapeutic importance. Both the relaxing effect on vascular smooth muscle and the effect on platelets are considered to be due to a stimulation of soluble guanylate cyclase by nitric oxide derived from the organic nitrate ester molecule through metabolization catalyzed by enzymes such as glutathione S-transferase, cytochrome P-450, and possibly esterases. The cyclic GMP **produced** by the guanylate cyclase acts via cGMP-dependent protein kinase. Ultimately, through various **processes**, the protein kinase lowers intracellular calcium; an increased uptake to and a decreased release from intracellular stores seem to be particularly important.

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ACCESSION NUMBER: 88075218 EMBASE
DOCUMENT NUMBER: 1988075218
TITLE: **Biosynthesis** of somatostatin in canine fundic D cells.
AUTHOR: Chiba T.; Park J.; Yamada T.
CORPORATE SOURCE: Department of Internal Medicine, The University of Michigan Medical School, Ann Arbor, MI 48109-0362, United States
SOURCE: Journal of Clinical Investigation, (1988) 81/2 (282-287). ISSN: 0021-9738 CODEN: JCINAO
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 023 Nuclear Medicine
029 Clinical Biochemistry
048 Gastroenterology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The observation that virtually all of the somatostatin-like immunoreactivity in the stomach consists of somatostatin-14 (S14), to the exclusion of somatostatin-28 (S28), suggests a unique pattern of prosomatostatin posttranslational **processing.** In order to examine the mechanisms by which S14 is **produced** from its precursor in the stomach, we investigated the **biosynthesis** of somatostatin in isolated canine fundic D cells. D cells pulse-labeled with [35S]**cysteine** revealed a cycloheximide inhibitable time-dependent incorporation of radioactivity into S14. A small fraction of radioactivity was incorporated into S28 but not into larger precursors. However, when the cells were incubated with monensin (1 μM), incorporation of radioactivity into a presumed somatostatin precursor was noted. Upon transfer of [35S]**cysteine** prelabeled cells to radioactivity-free medium, no conversion of S28 to S14 could be detected and the decrease of labeled S14 in cells correlated with a complimentary increase in the culture medium. Exogenous somatostatin inhibited somatostatin **biosynthesis** in a fashion that could be blocked by pertussis toxin pretreatment. Stimulation of prelabeled D cells with tetradecanoyl phorbol 13-acetate (10-7 M) of forskolin (10-4 M) for 2 h resulted in release of 41 and 33% of the newly **synthesized** radioactive S14, respectively, while only 9 and 6% of the total cell content of radioimmunoassayable somatostatin was secreted. These data

suggest that: (a) somatostatin is **synthesized** in fundic D cells primarily as S14, (b) S14 is **produced** by rapid **processing** of a larger precursor but there is little, if any, conversion of S28 to S14, (c) somatostatin **biosynthesis** is autoregulated, and (d) newly **synthesized** S14 is preferentially released from D cells in response to stimulation.

L20 ANSWER 14 OF 14 WPIDS COPYRIGHT 2004 THE THOMSON CORP on STN
ACCESSION NUMBER: 1987-049981 [07] WPIDS
DOC. NO. CPI: C1987-020945
TITLE: **Preparing** toxoid especially pertussis toxoid - by treating at least partially purified toxin with an oxidising agent to inactivate the toxin.
DERWENT CLASS: B04 D16
INVENTOR(S): SEKURA, R D
PATENT ASSIGNEE(S): (SEKU-I) SEKURA R D; (USSH) US DEPT HEALTH & HUMAN SERVICE
COUNTRY COUNT: 24
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 874637	A0	19861111	(198707)*		36
WO 8707507	A	19871217	(198751)	EN	
RW: AT BE CH DE FR GB					
W: AU BR DK FI HU					
AU 8778047	A	19880111	(198814)		
ZA 8704064	A	19880307	(198821)		
EP 269729	A	19880608	(198823)	EN	
R: AT BE CH DE FR GB IT LI LU NL SE					
US 4762710	A	19880809	(198834)		11
CN 87104279	A	19880120	(198909)		
JP 01500354	W	19890209	(198912)		
ES 2006763	A	19890516	(198944)		
IL 82825	A	19920525	(199225)		
CA 1302883	C	19920609	(199229)		
EP 269729	B1	19930331	(199313)	EN	21
R: AT BE CH DE FR GB IT LI LU NL SE					
DE 3785157	G	19930506	(199319)		
JP 2617500	B2	19970604	(199727)		11
KR 9610834	B1	19960809	(199923)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 874637	A0	US 1986-874637	19860616
WO 8707507	A	WO 1987-US1277	19870604
ZA 8704064	A	ZA 1987-4064	19870605
EP 269729	A	EP 1987-905002	19870604
JP 01500354	W	JP 1987-504462	19870604
ES 2006763	A	ES 1987-1759	19870615
IL 82825	A	IL 1987-82825	19870609
CA 1302883	C	CA 1987-539076	19870608
EP 269729	B1	EP 1987-905002	19870604
		WO 1987-US1277	19870604
DE 3785157	G	DE 1987-3785157	19870604
		EP 1987-905002	19870604
		WO 1987-US1277	19870604
JP 2617500	B2	JP 1987-504462	19870604

			WO 1987-US1277	19870604
KR 9610834	B1		WO 1987-US1277	19870604
			KR 1988-700186	19880215

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 269729	B1	Based on	WO 8707507
DE 3785157	G	Based on	EP 269729
		Based on	WO 8707507
JP 2617500	B2	Previous Publ.	JP 01500354
		Based on	WO 8707507

PRIORITY APPLN. INFO: US 1986-874637 19860616
AN 1987-049981 [07] WPIDS
AB US N6874637 N UPAB: 20011211

Preparation of a toxoid comprises treating at least partially purified or isolated toxin with an oxidising agent in an amount to chemically inactive the toxin while retaining the immunogenic property of the toxin, and recovering the intact toxoid or parts and **preparing** a vaccine.

Pref. oxidising agents are H2O2, sodium peroxide, N-chloro-4-methylbenzene sulphonamide sodium salt (chloramine-T), performic acid, dioxaneperoxide, periodic acid, sodium permanganate and sodium hypochlorite.

USE/ADVANTAGE - The treatment yields chemically irreversible antigen which is safe (non-toxic) without adverse effects encountered in prior art **prepns.**. The **preparation** is stable, immunogenic and protective against e.g. pertussis infection (whooping cough). The **method** can also be used for the **preparation** of other toxins such as tetanus, diptheria and cholera toxins.

Dwg.0/0

ABEQ EP 269729 B UPAB: 19930922

A **method** of **preparing** toxoid comprising the steps of treating at least a partially isolated protein toxin with an oxidant in the presence of a trace amount of metal ion to chemically inactivate the toxin whilst retaining its immunogenic property and recovering the inactivated toxin or components thereof.

0/3

ABEQ US 4762710 A UPAB: 19930922

Prepn. of toxoids comprises oxidn. of a protein toxin (opt. contg. impurities) at sites in the chain where **cysteine**, cystine, methionine, tryptophane and/or tyrosine units occur; and deactivation with transition metal ions, e.g. traces of Fe (II), Fe (III), Co or Cr, opt. in the presence of a chelating agent, e.g. EDTA.

USE - The **process** is applicable to bacterial toxins, e.g. **Bordetella pertussis** (whooping cough), and the prods. are components for improved vaccines having minimised side effects.